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THOMAS HUSTON MACBRIDE

B. SHIMEK

(WITH PORTRAIT)

The death of Thomas Huston Macbride at Seattle, Washington, March 27, 1934, removes one of the last of the pioneer mycologists of the Mississippi Valley, and one of the foremost students of Myxomycetes in the world. He was connected with the State University of Iowa for fifty-six years as Professor of Botany, President and President Emeritus, and was one of the associate editors of MYCOLOGIA from its foundation to the time of its adoption by the Mycological Society.

Thomas Huston Macbride was born in Rogersville, Tennessee, July 31, 1848. His father was a Presbyterian minister of strong abolition sentiments, and the family was compelled to leave Tennessee and came to Iowa several years before the outbreak of the Civil War. They located first near Salem and later in New London, Henry County; then in Cedar Rapids, which was then a small village; and finally in Princeton, Scott County.

He received his bachelor's and master's degrees in art from Monmouth College, Illinois, in 1869 and 1873, respectively, and later received honorary degrees from Monmouth, Lenox and Coe Colleges, and from the State University of Iowa. He served as professor of mathematics and languages in Lenox College, Iowa, from 1870 to 1878, when he was called to the State University as assistant professor of natural history. His college training was

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essentially in literary and linguistic fields, evidence of which was displayed throughout his life in the beauty and charm of both his written and spoken word.

His first preparation for his major life work was received on numerous field trips with his life-long friend, Dr. Samuel Calvin. The latter was a student, and also instructor in mathematics, in Lenox College, in 1864, when the former entered one of his classes in mathematics as a student. When Calvin left Lenox College in 1869 to take up his duties in the public schools of Dubuque, Iowa, Macbride succeeded him as instructor in mathematics, also teaching languages in addition. The field expeditions, which meant so much in the later activities of both men, were commenced in 1870, and continued during vacations for a number of years. These expeditions were devoted to the study of geology, botany and zoology, and on them both gained that broad, first-hand knowledge of these subjects which formed the basis for the development of the respective special fields in which both became eminent, and which gave to their teaching and research their characteristic breadth and balance.

On December 31, 1875, he married one of his former students at Lenox College, Miss Harriet Diffenderfer, who died May 28, 1927, after a happy companionship extending through more than half a century. One son, Philip D., and one daughter, Jean, survive.

In 1878 he was called to the State University of Iowa as assistant professor to Dr. Calvin, then occupying the chair (or as he later facetiously called it, the "settee") of natural history, from which were later evolved the departments of geology, botany, zoology and bacteriology. He early developed refined and advanced methods of plant histology and bacteriology when both subjects were in their infancy, and soon turned his attention to fungi and slime-moulds, which thereafter claimed his chief attention.

His first two extensive papers on fungi were published in 1888 in the "Bulletins from the Laboratories of Natural History of the State University of Iowa," then established through the efforts of Calvin and Macbride. The first of these was a report on the species of a portion of the genus *Agaricus*, then but little known in Iowa, and the second, a joint paper with Professor A. S. Hitch-

cock, on the Peronosporeae in Iowa, then equally little known. These papers were followed by others (see bibliography), but his attention was gradually drawn more and more to the Myxomycetes, on which group he became a world authority.

His first paper on slime-moulds, "The Myxomycetes of Eastern Iowa," was published in 1892, also in the University Bulletin, and attracted wide attention because the group was then almost unknown in the Mississippi Valley. This was followed by numerous other papers on the same general subject, the largest work being the standard monograph, "North American Slime-Moulds," the first edition of which appeared in 1899, the second, revised and enlarged, in 1922, and the third, which has been revised with the assistance of Dr. G. W. Martin, is in print. The collections of fungi and slime-moulds on which he based his studies are in the Herbarium of the State University of Iowa. A genus of the Hypocreales, *Macbridella,* several species of fungi and fossils, and a fern, have been named in his honor.

Macbride was a member of numerous scientific societies, and was entrusted by them with various official responsibilities. His pioneer contacts and early field experiences in primitive Iowa, coupled with his artistic temperament, early drew his attention to the need of preserving something of the original beauty and the natural wealth of his state, and he became distinctly the pioneer and the father of true conservation in Iowa. He was the founder of the Iowa Lakeside Laboratory on West Okoboji Lake, and in the past twenty-five years devoted much time and energy to the development of this inland station.

An eminent scientist, a profound scholar, an inspiring teacher, a brilliant public speaker, a public-spirited citizen, a lover of the beautiful in everything material as well as moral and ethical, a man of high character and a true friend, Dr. Macbride presented a rare combination of high qualities which made his well-rounded life an inspiration and a benediction.

His versatility is indicated by the variety of his papers and addresses. Throughout their wide range they show the influence of his early training and experience, and all are the scholarly expressions of a poetic and artistic nature. They treat of various subjects in general botany, mycology, paleobotany, geology, con-

servation and civic improvement, education, etc. Only the mycological bibliography is here presented.

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In the earlier papers the author's name appears as "McBride," but later he returned to the original Scotch form "Macbride."

AMANITA PANTHERINA OF WESTERN WASHINGTON

J. W. Hotson and Esther Lewis

(with plates 45 and 46 and 2 text figures)

In the fall of 1912 W. A. Murrill, during a visit in western Washington, described a new species of *Amanita* as *Venenarius pantherinoides* (*Amanita pantherinoides*) (**10**), closely related to *Amanita pantherina* Fries. This was prior to any definite knowledge that the latter species existed in North America. It was not until 1929 that Zeller (**17**) reported *Amanita pantherina* Fries as fruiting in the spring in Oregon. Since then attention has been focused on both spring and fall specimens, due to their striking appearance and abundance around the Puget Sound section.

Specimens were collected in the spring and again in the fall for detailed examination and comparison. This was not difficult as there was an abundance of material available, in season, around Fort Lewis, on the Tacoma "prairies," and in the vicinity of Seattle. It has also been reported from Grays Harbor, Anacortes, Olympia, and Mount Rainier. This species is found most abundantly late in the fall from November first until the frosts come. A specimen was collected Christmas Day, 1933 in the Nisqually Valley. It commonly occurs under young Douglas firs that have abundant branches near the ground, less frequently in the open. In some instances the spring and fall collections were from under the same tree. The following is a description of *Amanita pantherina* as it occurs in the Puget Sound region.

Pileus uniform Prout's brown (**15**) to warm buff (**15**) with a darker center, sometimes yellowish, especially around the margin, covered with white persistent scales often uniformly distributed but may be nearly or completely washed off by heavy rains (Fig. 2), 5–11 cm. broad, globose when young, expanded when mature, sometimes slightly depressed in mature specimens (plate 46), viscid when moist, shiny when dry, cuticle peeling readily; *margin*

incurved, obscurely striate, remnants of the veil sometimes adhering to it (PLATE 45); *flesh* white; *gills* white when moist, creamy when dried, adnexed then free leaving a line, sometimes sinuate, up to 1 cm. broad, narrowed behind, occasionally short and truncated, sometimes floccose; *stem* 7–15 cm. long, 1–2.5 cm. thick, white when fresh, creamy when dried, equal or attenuated upward, longitudinally fibrous, floccose to scaly upward, stuffed approaching hollow, viscid when moist, bulbous, bulb subspherical measuring 2–3.5 cm. in diameter, cortex tending to peel near volva (PLATE 45); *annulus* white, bell-shaped, prominent, superior, membraneous, persistent; *volva* white, distinct, circumsessile, often curved outward, occasionally with an extra ring; *spores* hyaline, granular when immature, 1–2 oil globules nearly filling the spore when mature, 10–12.5 by 7–9 μ, elliptical, smooth, obliquely apiculate (Fig. 1); *basidia* hyaline, 36–52 by 10–12 μ; *taste* mildly pleasant; *odor* faintly musty when fresh, somewhat nauseous when dried; *poisonous.*

FIG. 1. The obliquely apiculate spores of *Amanita pantherina.*

This description is nearly identical with Ricken's (**14**) except for the gill attachment. He states they are "free" and Rea describes them as "free, reaching the stem." Both fail to mention whether or not the spores are obliquely apiculate, but this characteristic is brought out in the spore illustrations given by Bresadola (**3**). The latter, also, gives illustrations showing that the gills reach the stem similar to PLATE 46. Spring and fall specimens were collected and sent to Zeller who stated that both were identical with what he described as *Amanita pantherina* from Oregon.

A comparison of the above description with that of *A. panther-*

inoides shows that the shape, size and color of the pileus are practically the same. The main differences are the sinuate gills, the glabrous stem and the smaller spores. In *A. pantherina* the gills are not always sinuate but are sometimes, the stem is never glabrous when young but may become so with age. The floccose character is quite evident when the specimens are carefully handled but it may disappear by rough usage or on drying. The greatest discrepancy is in the size of the spores. For *A. pantherinoides* these are described as "ovoid, smooth, hyaline $9 \times 5\,\mu$" (**10**). In the species under consideration the writers have found the spores to be larger. The measuremnts given were arrived at after measuring a large number of spores from specimens collected both in the spring and in the fall and they represent the best interpretation and conception of their size although some few were found larger and others smaller. Occasionally it was found that immature spores measured as low as $9\,\mu$. As a rule these immature spores could be detected by observing the spore-content which is granular while the mature ones have 1 or 2 oil globules nearly filling the entire spore.

The writers are convinced that the spring and fall forms which are found abundantly around the Puget Sound region are identical. Furthermore, we have not been able to find forms in which the size of the spores agree with those in the description of *A. pantherinoides* although the size of individual spores have approached these measurements. The spores of a young specimen collected by Zeller and identified by Murrill as *A. pantherinoides* and deposited in the herbarium of the Botany Department of the University of Washington in 1912, were compared with those of a similar specimen of *A. pantherina* collected at Fort Lewis, November, 1933. They were found to be identical in size, shape, and content.

Closely resembling *A. pantherina* are *A. cothurnata* Atk. and the *umbrina* variety of *A. muscaria* Fries both of which probably occur in Washington. The latter may be easily recognized by the yellowish flesh beneath the cuticle of the pileus and upper part of the stem. The former, however, is not so easily distinguished. The color of the pileus of *A. cothurnata* is usually white but sometimes tinged with a citron-yellow or tawny-olive in the center. It is specimens showing the latter characteristics that are hard to dis-

tinguish from similar forms of *A. pantherina*. The final distinction is largely based on the size and shape of the spores which are described as "globose, 8–9 μ in diameter. Beardslee (**1**) has questioned the accuracy of this statement, claiming that "The spores, which are at first ellipsoid, lose their cell contents and become filled with a large globule as described by Atkinson, and at the same time become inflated and globose."

THE POISONOUS CONSTITUENTS

The poisonous character of *A. pantherina* has been frequently demonstrated (**6**). An interesting case came to our attention during November, 1933, of three persons, a man and two women, who had eaten this fungus by mistake. The following statement was given by them shortly after their recovery:

Fifteen minutes after eating the mushrooms a dizziness came on with impaired vision, evidenced by the apparent remoteness of near objects. This was followed by hysteria, then a slightly intoxicated feeling which increased rapidly, and loss of coördination of mind, speech, and muscles. The man lost consciousness within fifteen minutes after ingesting the fungus, but the women never passed beyond a semi-conscious state. In this half-awake condition, one girl imagined herself in hell and back again, although without unpleasant reactions. "In fact," she said, "it didn't appear as bad a place as some people would have us believe." The other girl imagined herself released from her position and was somewhat worried until reassured. They said they felt certain death was approaching, but were without fear. Throughout the entire illness, no suffering was felt.

In general the symptoms of mushroom poisoning as exhibited by *A. pantherina* are those of organic muscarin such as is found in *A. muscaria*. This activating principle has been isolated from this species in Japan by Inoko (**4**) and from the European form by Boehm (**4**). In order to determine whether *A. pantherina* as it occurs in the Pacific Northwest contained organic muscarin an experiment was conducted for its isolation. The procedure was the same as that followed by Brieger and reported by Blyth (**2**).

One hundred grams of dried fungus was ground and macerated for three days in a liter of a solution containing one per cent of

hydrogen chloride in water. Toluol was added as a preservative. This solution was then centrifuged and filtered through a Buchner filter. The filtrate was placed over a steam bath, held at 60° C., under vacuum, and the excess moisture distilled off until a syrupy substance resulted. A ten per cent mercuric chloride solution was

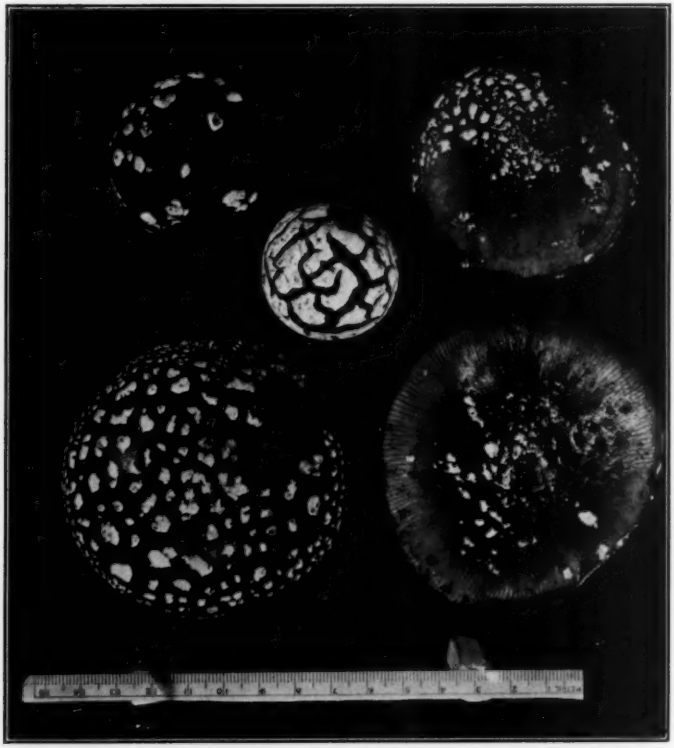

FIG. 2. *Amanita pantherina.* Showing variation in scales on the pileus.

added to this mushroom syrup, which was then centrifuged and filtered. The excess mercury was precipitated from the above filtrate by running through it an excess of hydrogen sulfide. It was again heated over a water bath of 60° C. and refiltered to remove the colloidal precipitate. A clear brown solution resulted. This was concentrated over a water bath at 60° C. under vacuum

to distill off the excess moisture. A liter of 95 per cent alcohol was added to the concentrated solution, which was then filtered, and the precipitate washed with alcohol. This was repeated using absolute alcohol. As much alcohol as possible was removed by distilling over a water bath at 35° C. under vacuum. The resulting solution, 290 cc., contained the active constituents from the 100 g. of the sample originally taken, which represents about a 34 per cent extract. Of this solution 20 cc. were taken to be used in testing for the presence of organic muscarin. To the remainder an excess of chloroplatinic acid was added and set aside to form crystals (2).

All the chemical tests made indicated the presence of organic muscarin. A precipitate was produced by auric chloride, potassiomercuric-iodide, phosphotungstic acid and phosphomolybdic acid; but no precipitate was formed with Lugol's solution, tannic acid, chloroplatinic acid, or picric acid. Another test showed that this solution containing the active constituents was capable of reducing copper sulphate to copper oxide and ferric chloride to ferric oxide. Organic muscarin, which is colorless and strongly alkaline, is readily soluble in water and alcohol but sparingly so in chloroform and is insoluble in ether.

A further test was made to determine the effect on a frog's heart. When 1 cc. of the mushroom solution was injected subcutaneously into the back of a frog, paralysis resulted within ten minutes but no apparent action on the heart was noticed. Then 2 cc. were injected in a second frog with similar results except that paralysis occurred in three minutes. The fact that the 2 cc. contained less than .7 g. of the original active constituents would probably explain why there was no apparent heart reaction. The solution was then applied directly to the heart which stopped beating within four minutes. A one per cent solution of atropin was then applied, with no immediate effect until a weak electric current was used as a stimulant, then the heart slowly resumed its beating. Another frog was prepared and the heartbeats counted, 62 beats per minute. The extract was again applied directly to the heart and in three and a half minutes it started to miss beats; in four and a half minutes it stopped. A one per cent solution of atropin was applied, and in a few seconds it started pulsating. A weak

electric current was applied and in five minutes the heart was beating at the rate of twenty-eight beats per minute, and continued at this rate for forty-five minutes. A fourth frog was prepared and the extract applied to the heart which was arrested in diastole in four minutes; then two drops of a three per cent atropin solution were administered to the thus arrested heart. The heart slowly resumed beating without the stimualtion of a weak electric current. On the application of this mushroom solution directly to the heart the action is noticeably decreased, with lessened systolic and increased diastolic excursions, and finally stoppage in diastole. This is characteristic of the action of organic muscarin on the frog's heart (**16**). Large doses paralyzed the vagus and ganglia. Reflex action was perfect throughout the experiment. The conclusion is that organic muscarin is present in small amounts in *A. pantherina* of the Pacific Coast.

The writers are deeply indebted to Fred F. Johnson, assistant state chemist, for valuable assistance in extracting the muscarin; to Dr. E. Victor Smith for advice and coöperation in the experiments with the effect of muscarin on the frog's heart; and to D. E. Stuntz for valuable assistance with the photographs.

SUMMARY

1. *Amanita pantherina* Fries occurs in Washington. It has been reported from Seattle, Olympia, Mount Rainier, Fort Lewis, Tacoma, Anacortes, and Grays Harbor.
2. The writers believe that *Amanita pantherina* Fries and *A. pantherinoides* Murr. are identical.
3. Several cases of mushroom poisoning have resulted from eating this species.
4. At least one of the active principles in this mushroom is organic muscarin. This was isolated and tested.

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HERMAPHRODITISM INVOLVING SELF-STERILITY AND CROSS-FERTILITY IN THE ASCOMYCETE PLEURAGE ANSERINA [1]

L. M. AMES [2]

(WITH 6 TEXT FIGURES)

For many years the study of sexuality of fungi has been carried on with increasing interest by many mycologists and plant pathologists. By them many different sexual conditions have been discovered among the various groups of fungi, and several theories have been offered to explain these sex phenomena. Much of this work has been done in the Ascomycetes which have furnished material very suitable for such study. Recently, in *Pleurage anserina* (Ces.) Kuntze, one of the Sordariaceae, the asci of which normally contain four binucleate spores, occasionally one or two dwarf uninucleate spores and rarely one giant multinucleate spore, the writer has found that the occasional uninucleate spores when germinated developed a mycelium which is hermaphroditic and self-sterile, but reciprocally fertile with similar but compatible cultures. This situation has already been noted in a brief preliminary paper. Subsequent work has added further knowledge concerning the sexuality both of the normal, customary, binucleate spores and of the abnormal, rarely occurring, multinucleate spores. A more complete discussion concerning the sexual condition of *P. anserina* is offered in this paper.

PREVIOUS WORK ON THE SEXUALITY OF PLEURAGE ANSERINA

The development of the archicarp of *P. anserina* has been observed by Wolf (**8**), who found in this species (which he called

[1] Contribution from the Laboratories of Cryptogamic Botany, Harvard University, No. 131.

[2] The writer wishes to express his appreciation to Dr. Wm. H. Weston, Jr., for stimulating encouragement and helpful criticism during the progress of this investigation and the preparation of the manuscript.

Podospora anserina) that the perithecia arise from hyphal coils, and suggested that the coiling together of the two hyphae very probably represents the sexual process.

More recently, a condition of homothallism was reported for this fungus by the writer (**1**) as the result of experiments done during 1927–1929, with single spore cultures from the normal binucleate ascospores which occur four in an ascus. Each culture derived from a single spore, growing independently, produced many perithecia, and such a condition was interpreted to indicate that such a culture was bisexual and self-fertile (homothallic). The small uninucleate spores were observed at this time but work on them was deferred to a later date.

Subsequently Dowding (**5**) reported that the normal binucleate and giant multinucleate ascospores produced mycelia which were homothallic and that the mycelia from the dwarf uninucleate spores occasionally developed in the asci were heterothallic "unisexual." In the same article, she points out also that the mycelia from the normal spores bore no kind of secondary spore which might be a possible means of mixing cultures.

The writer, however, found the sexual situation in *Pleurage anserina* to be much more complex than the previous studies and reports gave evidence. In a preliminary paper (**2**) he showed that spermatia as well as coil-like ascogonia were produced in the cultures developed from uninucleate spores. Experimental results were reported in this preliminary paper showing that these cultures from single uninucleate spores instead of being heterothallic "unisexual," as reported by Dowding, were in reality bi-sexual and self-sterile, and that the female and male organs were morphologically distinct. Furthermore it was shown that reciprocal fertilization took place between certain of these cultures derived from the unnucleate spores; such cultures were complementary, the difference between them being not a difference of sex, because both sex organs were on each, but a difference of compatibility.

MATERIAL AND METHODS

The material of *Pleurage anserina* was first collected from horse dung at East Lansing, Michigan, in the fall of 1927, and later from the dung of various herbivorous animals in the vicinity of

Cambridge, Mass. As the cultures from the two sources are the same species and identical in all of their sexual reactions there has been no attempt in this paper to make a distinction between them.

The fungus is well adapted to the study of sexuality because the sex organs are morphologically distinct, it will develop with extreme ease in cultures, and a complete generation can be grown in approximately three weeks. The chief advantage, however, lies in the fact that within the asci which normally contain four binucleate spores, occasionally three binucleate spores and two uninucleate spores will form. These uninucleate spores enable one to obtain cultures in which there is only one type of nuclei. The importance of these uninucleate spores will be brought out later.

The media upon which *P. anserina* has been cultivated were, chiefly, potato dextrose agar and prune agar, on which the fungus grows and fruits normally. These media are of special value because, for the most part, on them the fungous mycelia remain light colored while the media remain clear, thus permitting the undisturbed ascogonia as well as the mycelia to be carefully examined with a microscope with light transmitted from beneath the Petri plate or slide. Other culture media were tried, such as dung agar, oatmeal agar, synthetic agar, etc., but because the fungus either turned them dark or would not grow well, their use was discontinued.

Single spore cultures of *P. anserina* were obtained by isolating the spores with a spatula made by grinding down a No. 10 sewing needle and inserting it in a slender handle of soft wood. After crushing a perithecium with a cover glass, the asci, as seen under the microscope, were usually spread out in a fan-like formation. If one was noticed that contained an abnormal number of spores or was for any reason desired, the cover glass was tapped carefully until that particular ascus was separated from the others. The cover glass was then slipped off and the isolated ascus allowed to become dry or almost dry. Then with the spatula, the spores were picked off in the order in which they occur in the ascus and planted separately on plates of agar. This method of obtaining single spores, besides being rapid is fairly easy.

For special study, subcultures were made on slides coated with

a thin layer of clear agar. These were prepared by pouring hot agar over sterile slides in Petri plates, so that a thin film of agar covered the slide, and the remaining agar covered the bottom of the plate and aided in keeping the culture moist and in supplying enough material for normal growth. In such subcultures from uninucleate ascospores, the sex organs as well as the mycelial growth were easily observed under a microscope while the cultures were illuminated from beneath with transmitted light.

For the working out of fertilization, the following methods were used. The sexual organs usually began to appear on these thinly coated slides about six or eight days after the inoculum had been transferred to them. After the sex organs appeared, spermatia from a compatible culture were placed upon the ascogonia either by smearing them over groups of ascogonia or by placing spermatia on particular ascogonia. The gross smears were made with a sterilized camels hair brush, on some dishes. On other dishes, the spermatia were placed on particular ascogonia with a sterilized rat whisker, used because it was flexible and therefore would not injure the delicate structures.

In the preparation of permanent slides showing the fungus in its different stages of development, cultures were flooded with killing and fixing solutions, the air was exhausted with a suction pump; the fungus was thus killed in its natural, undisturbed condition. Then the slides were removed from the Petri plate with its thin agar coat impregnated with the fungus and dealth with in a manner similar to that used for serial sections. In the latter steps, great care was needed in order to carry the film of agar through the alcohols and clearing solutions without injury. The most satisfactory stain was iron alum haematoxylin. During the process of staining it was found that even though the excess stain washed out of the agar with some difficulty, the agar became sufficiently clear to study the stained fungous parts.

GENERAL MORPHOLOGY

The details of morphology and the cultural characteristics of the fungus were determined by the study of the material in both the living and in the fixed conditions. The perithecia of *Pleurage anserina,* growing naturally on dung, may be half sunken or may

be entirely superficial, may occur scattered quite uniformly or may be aggregated in small clusters, while on agar media the perithecia range in position from totally submerged to superficial. The perithecia are membranous but not transparent, the neck being characterized by clusters of stiff, bristle-like hairs. The asci within the perithecia usually contain four binucleate spores, but occasionally asci may contain abnormal numbers of spores ranging from giant multinucleate spores to five spores, of which three are

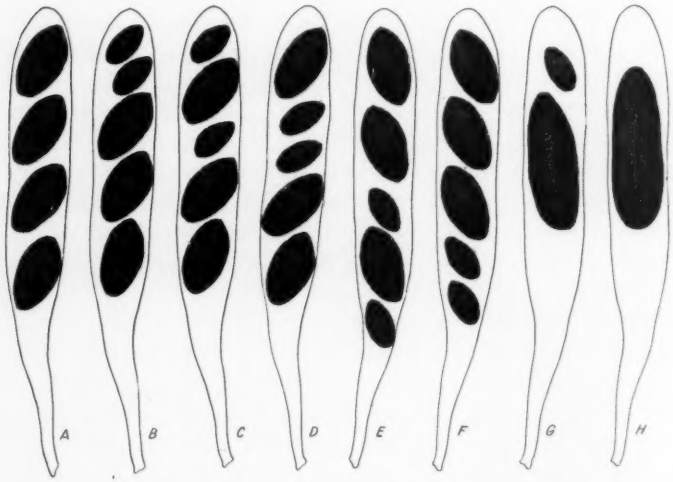

FIG. I. Diagram of asci of *Pleurage anserina* showing some variations in number and type of spores at maturity. The normal 4 spored condition (A); the condition of three normal and two small spores occasionally encountered (B–F); one large and one dwarf spore (G); and one giant spore (H).

normal binucleate and two dwarf uninucleate spores (FIG. I, B to H).

The spores of *P. anserina* are uniseriate in arrangement within the ascus, elliptical in shape, and in color range from hyaline when young through olivaceous to dark brown and opaque when mature. The normal spores range in size from 16–20 $\mu \times$ 32–42 μ, and extend below into a short, hyaline, primary appendage, 1–1½ times the length of the spore, terminated by a lash-like, gelatinous,

secondary appendage of variable length, while the apex of the spore is terminated by a similar, gelatinous appendage and in the wall at the apical end of the spore is situated a small germ pore. The formation of the spores in the ascus proceeds as follows: at first the young spore is a small allantoid cell, the contents of which do not differ materially from that of the ascus. The cell grows for a while in all dimensions, but soon all of its protoplasm congregates in and enlarges the upper end, while a septum develops separating this swollen portion, which becomes the actual spore, from the empty remainder, which becomes the primary appendage. These stages of spore formation, as observed by the writer, have been found to agree with those already described by Griffiths (**6**). The young spores in the hyaline condition, or just slightly olivaceous, are readily stained with lactophenol-cotton blue which allows the nuclei to be seen. The normal spores are binucleate, the dwarf spores are uninucleate, and the giant spores are multinucleate. This relation of spore and nuclear number in this fungus has already been noted by Wolf (1912) and discussed by Dowding (1931).

The spores germinate by the protrusion of a bulbous enlargement (FIG. II, 9), through the germ pore at the apical end; from this enlargement a few main hyphae come out, from which the mycelia arise. In general, the life cycle from beginning of mycelial growth to the formation of mature ascospores requires about three weeks at laboratory temperature. The life cycle, under most favorable conditions on dung, however, may be completed in 9 to 14 days.

The development of cultures derived from uninucleate ascospores gives an opportunity to study the morphology of the fungus and to determine the fundamental facts concerning the sex organs and their relationship to each other. Such cultures derived from uninucleate spores grow normally in every respect, but even after an interval of more than a month fail to produce perithecia. Those cultures do, however, produce both ascogonia and spermatia in abundance.

The antheridia may be solitary or associated in groups usually without regularity in position. The antheridium (FIG. III, 6, 7, 8) is usually a more or less bottle-shaped outgrowth of a cell, some-

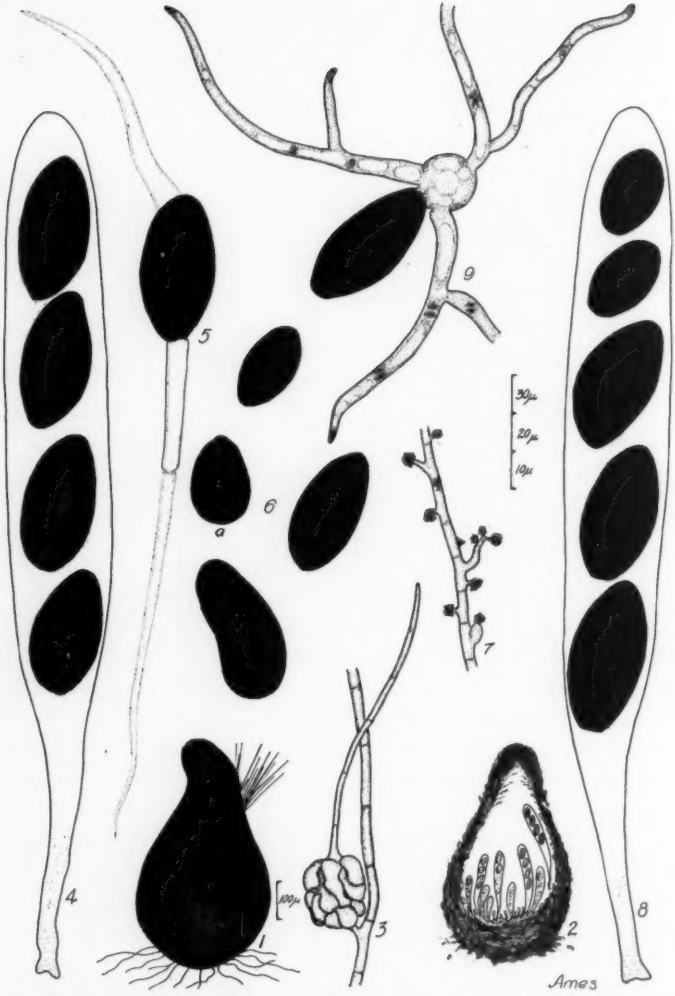

FIG. II. *Pleurage anserina* (Ces.) Kuntze. 1, habit of mature perithecium; 2, median longitudinal section of a perithecium; 3, young ascogonium with trichogyne; 4, mature ascus containing four normal binucleate ascospores; 5, ascospore showing gelatinous appendage which is characteristic of the spores of this species; 6, group of ascospores showing variation in size and shape, 6a, spore showing germ pore; 7, mycelial branch bearing antheridia with clusters of spermatia; 8, mature ascus showing three normal and two dwarf spores; 9, late stage of the germination of an ascospore.

times flask-shaped, variable in the diameter of the base, in the total length and in shape; the form and size is not constant, but the range of variation is, nevertheless, not great. In length, the antheridia range from approximately 3 μ to 5 or 8 μ; each antheridium being differentiated, more or less abruptly, into a basal somewhat broad or sometimes inflated portion (FIG. II, 7) which usually tapers to a narrow apex in which there is a short canal through which the individual spermatia are budded off successively.

The spermatia, which usually begin to appear about 6 or 8 days after ascospore germination, arise by the protrusion, through what appears to be a constriction just below the discharge pore of the antheridium, of small, uniform, uninucleate portions of protoplasm which, after emergence, becomes spherical, and the dense nucleus in each is surrounded by a transparent envelope. The spermatia are approximately 2 μ in diameter, and the enclosed nucleus measures a micron or slightly more in diameter. A single antheridium may give rise to a few or to a cluster of many spermatia. The spermatia may be transported by various means, such as air currents, water, insects, etc. The clusters of spermatia are so loosely associated that a slight disturbance may dislodge and scatter them.

The female fundaments (ascogonia) usually appear about 6 or 8 days after spore germination, and are formed from side branches on the more mature parts of the mycelium, that is, back from the rapidly growing margin of the culture; they may be submerged in the substrate or totally superficial. The ascogonia seem, for the most part, to be formed from cells which send out short, swollen branches, the contents or which are dense, and stain deeply. From observations on numerous ascogonia, it seems evident that from these enlarged, short branches others cells are produced whose contents are almost transparent in contrast with the deeply staining ones. These clear cells which in turn branch and rebranch, wrap themselves tightly about the initial dark staining cells, thus giving the appearance of a coil-like ascogonium. One of the initial branches, however, grows away from the ascogonium (FIG. II, 3), and is interpreted as a trichogyne, because it has been observed that when compatible spermatia were brought in contact with it, fertilization took place and then the ascogonium developed

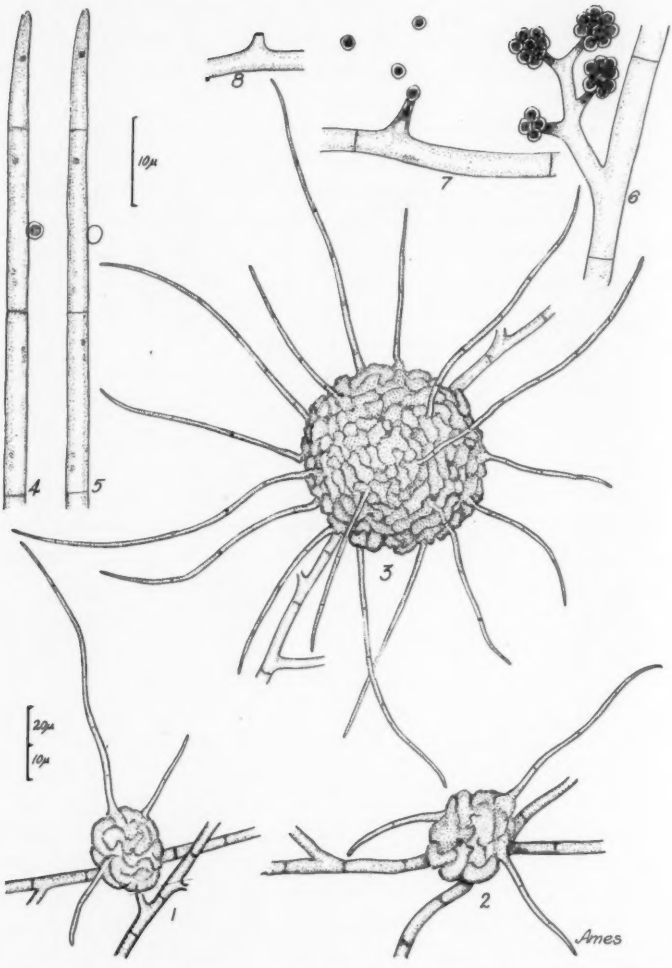

FIG. III. *Pleurage anserina* (Ces.) Kuntze. 1–3, showing different stages of development of ascogonia in which fertilization has not taken place; 4, receptive hypha showing attached spermatium; 5, receptive hypha showing an empty spermatium remainder; 6, spermatia clustered at antheridial tips; 7, emergence of a spermatium from an antheridium; 8, empty antheridium showing aperature.

into a perithecium. Very often radiating from the young ascogonium there are secondary receptive branches which also function as trichogynes. At this stage of development, the ascogonium normally becomes fertilized. If fertilization does not occur, however, the ascogonium slowly increase in size, sometimes to as much as 50 or 75 μ in diameter, and at the same time send out more receptive branches until, in some cases, these may increase to as many

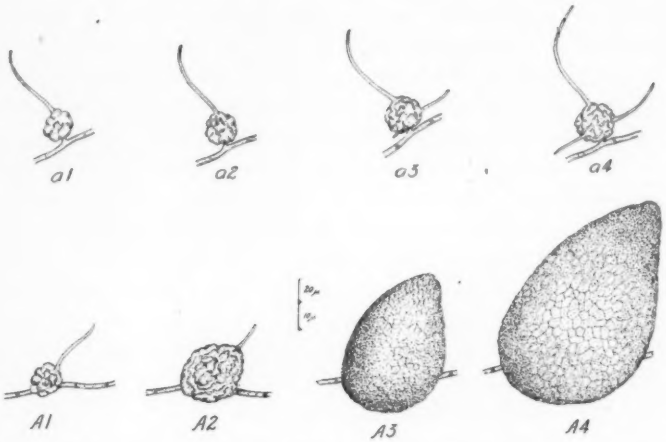

FIG. IV. *Pleurage anserina* (Ces.) Kuntze. Drawings made by aid of camera lucida showing stages in the development of two ascogonia. a, ascogonium in which fertilization has not taken place, A, ascogonium in which fertilization has taken place.

a2, A2 at the end of 36 hours.
a3, A3 at the end of 72 hours.
a4, A4 at the end of 96 hours.

as 40 or 50 in number (FIG. III, 3). The receptive hyphae are composed of a variable number of cells, usually from 2 or 3 to 8 or 10 cells. If fertilization does take place in an ascogonium it develops rapidly into an organized parenchymatous structure, spherical at first but soon forming a neck due to unequal growth at the apex (FIG. IV); the perithecia are pear shape at maturity, and bear on the convex surface (FIG. II, 1) of the neck tufts of long dark brown, sparsely septate hairs.

EXPERIMENTAL WORK

The formation of perithecia in *Pleurage anserina* involves a situation more complicated than might have been assumed from previous work reported for this and for other ascomycetes. In the cultures from uninucleate spores the condition of hermaphroditism was first suspected in the fall of 1930, as the result of an accident. In one Petri dish two complementary mycelial cultures were growing, each culture was derived from a uninucleate ascospore, and a line of young perithecia had already formed along the margin of their intermingling hyphae; at this stage the writer noticed a small mite crawling on one mycelium and in spite of the fact the plate was considered spoiled the writer, through curiosity, watched the progress of the mite's wanderings from day to day as it made a tortuous path from one mycelium over the other. After several days it was noticed that perithecia were beginning to grow in the path made by the mite, and in two weeks there was no question of this because the tortuous trail was well defined by mature and maturing perithecia. This observation led to the assumption that the mite had transported something on its body from one mycelium to the other. A microscopic study disclosed that in addition to coil-like ascogonia from which, after fertilization, the perithecia develop, there were produced on short branches minute spores which looked suspiciously as if they might be functional spermatia. This suggested that it was probably these minute bodies rather than bits of mycelium which had been transported by the mite, and that even the line of perithecia along the intermingling margin of the two mycelia probably had resulted from the fertilization of the ascogonia by these bodies rather than by hyphae. Following the clue suggested from the results of observation of the mite, and from preliminary microscopic observations of the fungus, many more single uninucleate ascospore cultures were examined, and each was found to produce both ascogonia and spermatia; yet these cultures derived from uninucleate spores never, independently, produced perithecia, even though both the ascogonia and spermatia were present, and even though many were in contact which thereby provided ample opportunity for self-fertilization. It seemed probable, therefore, that the asco-

gonia and microspores were, respectively, funtional female and functional male bodies, but that on the same mycelium they were self-sterile, while from one mycelium to another they would be cross-fertile. Accordingly tests were made to settle this point.

When, between complementary cultures, spermatia of one were transferred to the ascogonia of the second, and spermatia of the second were transferred to the ascogonia of the first, fertilization took place and perithecia were developed on both cultures (FIG. V). Reciprocal crosses have been made using innumerable spermatia streaked over many ascogonia, and reciprocal crosses have been made in which one particular ascogonium in each culture was under observation; fruiting bodies developed only from those ascogonia that had been fertilized with spermatia from complementary cultures.

Between 250 and 300 single uninucleate spores were isolated and the cultures derived from them studied; these were found to fall into two classes, which can be designated as class *A* and class *B*, in a ratio of 1 : 1. All cultures derived from single uninucleate spores belonging to class *A* are self-sterile and reciprocally inter-sterile; all cultures derived from single uninucleate spores belonging to class *B* are self-sterile and reciprocally inter-sterile; but any one or all cultures derived from single uninucleate spores of class *A* are reciprocally fertile with any one or all cultures derived from single uninucleate spores of class B. From the many experiments performed and the microscopic examinations made, it was now clear that the difference between the cultures derived from uninucleate ascospores designated above as class *A* and class *B* is not a difference of sex but represents a difference of compatibility.

It seemed imperative at this time to find out specifically how fertilization was initiated and what part or parts of the fungus entered into the sexual act. Accordingly the following experiments were performed. In one case a compatible spermatium was placed in contact with a primary receptive hypha, three cells from its tip, of a very small ascogonium which had not produced secondary receptive hyphae, and whereas the spermatium was observed to be filled with dense contents when first in contact, later the spermatium was found to be empty. It is assumed, therefore, that even though the actual migration of the spermatium nucleus

has not been observed, the nucleus passed down the receptive hypha and reached the essential female cell, because the ascogonium promptly developed into a perithecium with normal asci and ascospores. In another instance the spermatium was placed two cells distant from the ascogonium, in contact with the primary receptive hypha; the spermatium was observed to be filled with dense contents when placed in contact with the receptive hypha, and later when observed, the spermatium was found to be empty; the ascogonium in a few days developed into a mature perithecium. In still another case, spermatia were placed on the coil-like portion of the ascogonium; the contents of the spermatia could not be observed in this instance, but fertilization must have taken place because of the prompt development of the ascogonium into a mature perithecium. Compatible spermatia have been placed in contact with the mycelium adjacent to the ascogonium in several cases, but the spermatia remained the same, retaining their dense contents and the ascogonia did not develop. Ascogonia from cultures derived from uninucleate spores have had mycelia from a compatible culture in contact with their receptive hyphae, but no evidence of fertilization followed. In the same way, when compatible spermatia were placed in contact with one another, no fusion nor growth was seen to occur; and likewise compatible ascogonia in contact did not initiate fertilization.

Spermatia from incompatible cultures were also brought in contact with mycelia in the attempt to cause fertilization, but the spermatia lost none of their contents showing their inability to effect fertilization and as might be expected no perithecia developed. Likewise in any one culture from a uninucleate spore, the ascogonia were in no way affected by the spermatia from the same or other incompatible cultures. The spermatia from this source, when in contact with receptive hyphae of the ascogonia, remained unchanged, and the ascogonia did not develop into perithecia.

The male and female organs from any one culture derived from a uninucleate spore, were, therefore, shown to be incompatible, and further it was shown that fertilization takes place only when spermatia and ascogonia from separate but compatible cultures come in contact. It has therefore been concluded that cultures derived from uninucleate spores are structurally hermaphroditic, in that

they produce both male and female organs, but the behavior of these male and female organs gives evidence that, although they are self-sterile they are also each functional, and that there resides in the nuclei of these uninucleate spores the genetical qualities involving a difference of compatibility instead of a difference of sex.

Although it has not been possible, as yet, to trace the migration of the male nucleus, or to determine cytologically the part it plays in fertilization yet some points may be assumed from the experimental evidence. If, as some might argue, the spermatium nucleus plays no part in effecting fertilization, and if this process is accomplished through the fusion of various nuclei originating in the ascogonium, then we would expect to find production of perithecia in cultures derived from uninucleate ascospores. As already stated, however, the experiments demonstrate that such cultures are self-sterile and never do produce perithecia. From the experiments, also, we find that in these cultures from uninucleate spores, two classes of compatibility are involved. The production of perithecia follows the bringing together of two such compatible cultures. Furthermore experimental evidence indicates that these qualities of compatibility are inherent in the nuclei. If then, the spermatia from a culture of one compatibility do indeed take part in the fertilization of the ascogonia of the culture of the other compatibility, we would expect to find in the distribution of the nuclei in the ascospores of the perithecia that develop after this fertilization, a segregation of the two classes of compatibility in a ratio of 1 : 1. The experiments which have been carried out indicate that this is indeed the case. It seems justifiable, therefore, to assume that fertilization is accomplished by the fusion of ascogonial with spermatial nuclei, and not by the fusion of paired ascogonial nuclei.

After working out the sexual situation of the cultures derived from the uninucleate ascospores, however, and finding that they fall into two groups which differ from each other, not in sex, but in compatibility, and after having assumed that the quality of compatibility resides in the nucleus and is segregated in a ratio of 1 : 1, the writer was led to the question of the status of the normal binucleate spores. Previously the binucleate spore cultures of *P. anserina* were reported by Ames (1930) and by Dowding (1931)

to be homothallic because the mycelium from a single spore produced perithecia. It was assumed at that time that the mycelia from such single spore cultures were necessarily bisexual and self-fertile (homothallic), because they produced fruiting bodies when grown alone.

From the behavior of the cutlures from the uninucleate ascospores, it seemed possible to the writer that the mycelia produced from the normal binucleate ascospores were, in reality, not homothallic, *i.e.*, simply bisexual and self-fertile, as they were previously thought to be, but gave only the appearance of homothallism. Experiments were carried on to see if this were true, with the following results.

If the binucleate ascospores really contain two compatible nuclei, it seemed possible that by isolating many hyphal tips some of them would contain nuclei of only one class of compatibility. As a means of studying the sexual condition of the normal binucleate ascospores, some of the spores were isolated on agar, and shortly after germination had begun, hyphal tips were isolated. By this means six asci were separated from three perithecia and of the 24 spores isolated twenty germinated. From the mycelia derived from these twenty ascospores, 207 isolations were made. Those isolations fell into three groups; in the first group *A*, 23 isolations which, when grown by themselves or grown among themselves, remained without fruiting; in the second group *B* there were 19 isolations, which, when grown by themselves or grown among themselves, remained without fruiting; but any one or all of the first group A, were reciprocally fertile with any one or all of the second group *B*. The crossing experiments, as well as the microscopic examinations, show that the two groups, *A* and *B*, are each hermaphroditic and self-sterile, similar in every respect to the two groups derived from the uninucleate spores with which they were further tested. In the third group, *C*, there were 165 isolations which, when grown separately, produced perithecia. It was obvious that in each isolation of group *C* there must have been present nuclei of both types of compatibility.

On the basis that two compatible ascospores differ from each other only in respect to compatibility, and on the basis that the genetical quality of compatibility resides in their nuclei, it is as-

sumed, therefore, that the two nuclei in the normal ascospores differ from each other, not in sex, but in respect to compatibility. This assumption is borne out by the fact, as has already been shown, that it is possible to separate these two types of nuclei, from the mycelium derived from germinated binucleate spores, by means of hyphal tip isolations. The cultures derived from the normal binucleate ascospores of *P. anserina,* therefore, only appear

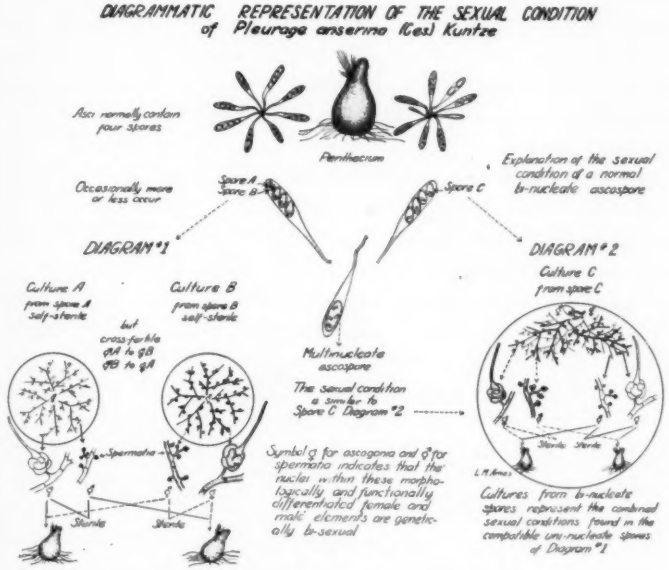
DIAGRAMMATIC REPRESENTATION OF THE SEXUAL CONDITION
of Pleurage anserina (Ces) Kuntze
Asci normally contain four spores
Perithecium
Explanation of the sexual condition of a normal bi-nucleate ascospore
Occasionally more or less occur
Spore A
Spore B
Spore C
DIAGRAM °1
DIAGRAM °2
Culture A from spore A self-sterile
Culture B from spore B self-sterile
but cross fertile ♂A to ♀B ♂B to ♀A
Culture C from spore C
Multinucleate ascospore
The sexual condition is similar to Spore C Diagram °2
Spermatia
Sterile
Sterile
Sterile
Sterile
Symbol ♂ for ascogonia and ♂ for spermatia indicates that the nuclei within these morphologically and functionally differentiated female and male elements are genetically bi-sexual
Cultures from bi-nucleate spores represent the combined sexual conditions found in the compatible uni-nucleate spores of Diagram °1

FIG. V. *Pleurage anserina* (Ces.) Kuntze. Diagrammatic representation of the sexual condition in the hermaphroditic self-sterile cultures derived from the uninucleate ascospores A and B of *P. anserina,* and the production of perithecia by cross-fertilization of compatible strains. Diagram C of culture C shows the sexual condition of mycelia derived from normal binucleate ascospores.

to be homothallic, that is hermaphroditic and self-fertile; but in reality, as the data disclose, there are two hermaphroditic cultures present growing intermingled as one, each giving rise to both male and female organs which are self-sterile, but which are reciprocally fertile (FIG. V). This condition is quite different from that defined as homothallism.

In the case of the giant multinucleate ascospores also the sexual situation was found to be essentially like that described for the normal binucleate ascospores, except that more initial nuclei are concerned, eight nuclei being enclosed in a single giant ascospore. The sexual nature of the mycelia derived from the giant spores was studied in a way similar to that just described for the normal spores. Three multinucleate ascospores were isolated, and, shortly after germination, 62 hyphal tips were cut off and transplanted to separate plates. The development of the cultures from the hyphal tip isolations showed that they also fell into three groups. In the first group A, there were six isolations which, when grown by themselves or grown among themselves, remained without producing perithecia; in the second group *B*, there were eight isolations, which, when grown by themselves or grown among themselves, remained without producing perithecia; but any one or all cultures of the first group *A*, were reciprocally fertile with any one or all of the second group *B*. The crossing experiments, as well as microscopic examinations, show that the two groups, *A* and *B*, are each hermaphroditic and self-sterile, similar in every respect to the two groups derived from the uninucleate spores with which they were further tested. In the third group *C*, there were 48 isolations which, when grown separately, produced perithecia. It is apparent that in each isolation of group *C*, there must have been present nuclei of both types of compatibility.

In contrast to the usual distribution of nuclei in the ascospores of *P. anserina*, there are occasionally exceptional cases of nuclear distribution. From the study of the normal binucleate ascospores thus far discussed it would be expected that the mycelia from all binucleate ascospores will produce perithecia when grown by themselves. From the diagram (FIG. VI, *A*) it is seen that all normal ascospores contain two nuclei which are compatible; occasionally, however, the nuclear distribution may vary, since it was found to be possible for two nuclei which are of the same compatibility class, and therefore inter-sterile, to be enclosed in a single, apparently normal, ascospore. Because the nuclei are of the same compatibility class, the ascogonia and spermatia produced by the mycelium of such a spore will be self-sterile.

Of all the binucleate ascospores grown, the writer has encoun-

tered only one case in which the mycelium from a binucleate ascospore failed to produce perithecia when grown by itself. In this one case, the ascus studied had three binucleate spores and two

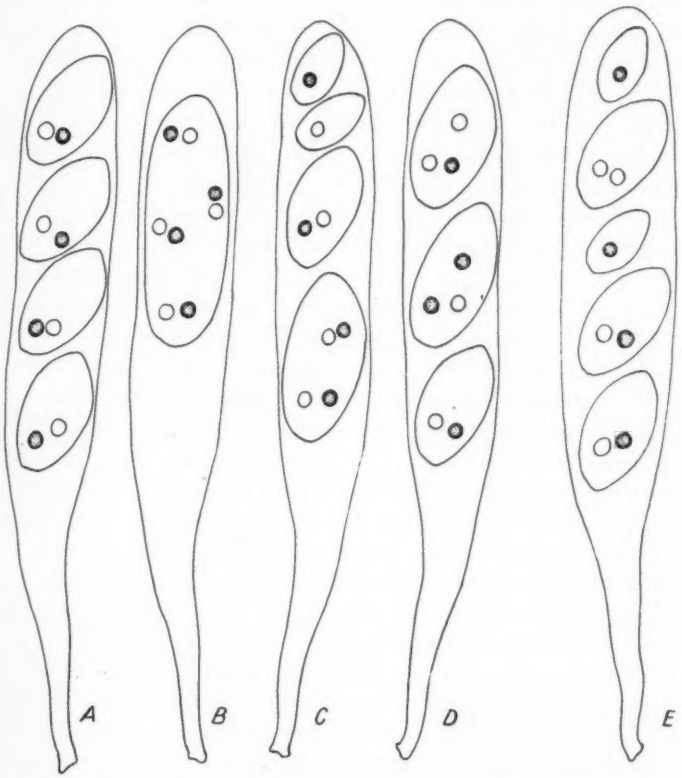

FIG. VI. *Pleurage anserina* (Ces.) Kuntze. Diagram of asci with various numbers of spores showing their respective nuclear condition. A, normal binucleate spores; B, C, D, nuclear distribution in abnormal spores; E, an unusual case in which an ascus contains two normal binucleate spores, one binucleate spore containing two nuclei of the same compatibility, and two uninucleate spores of the same compatibility.

dwarf spores, arranged from stipe to apex (FIG. VI, *E*), thus: two binucleate spores, one dwarf spore, one binucleate spore, and the final dwarf spore. These were picked off in the order of occur-

rence and grown separately on agar. The binucleate ascospore which formed between the two dwarf ones, grew normally in every way, produced abundant ascogonia and spermatia, but never produced perithecia. The mycelia from the two dwarf spores, when mated together, did not produce perithecia. However, reciprocal crosses between either of the cultures from the dwarf spores with the culture from the binucleate spore resulted in the production of perithecia. This shows that the nuclei must have been arranged so that this one spore enclosed two nuclei of the same compatibility class, and therefore was inter-sterile, and that each of the dwarf spores contained a nucleus of the same compatibility class, and were likewise inter-sterile. The other two binucleate ascospores gave rise to cultures each of which produced perithecia when grown by themselves, and hence contained the normal distribution of compatible nuclei. The segregation of nuclei in the development of the ascospores of *P. anserina* may probably be explained in the same way that Dodge (**4**) has shown for the nuclei of *Neurospora tetrasperma,* except that instead of a segregation of sex as shown for *Neurospora,* there is in *Pleurage anserina* a segregation of compatibility; the two types of nuclei may be segregated equally well in the first, second, or third divisions and become arranged in such a manner that each normal spore will contain a pair of compatible nuclei.

The experimental evidence shows that the ascospore nuclei of *P. anserina* fall into two distinct classes, *A* and *B*, in a ratio of 1 : 1. The nuclei in the mycelium of each class have the power to initiate the formation of both ascogonia and spermatia which, even though self-sterile, are nevertheless each functional. It is seen that each class does not represent a difference of sex, but represents a difference of compatibility.

Because it has been found that cultures from uninucleate spores of *P. anserina* are bisexual self-sterile but cross-fertile, it is no longer justifiable to conclude that a culture from a single spore is unisexual simply because it does not produce fruiting bodies when grown by itself and because fruiting bodies are initiated by mating complementary cultures; likewise it is no longer justifiable to conclude that a culture is simply bisexual and self-fertile because it does produce fruiting bodies when grown by itself.

It has been noted in a general way, no accurate counts being made, that the relative number of ascogonia formed on cultures from class *A* are approximately equal in number to the ascogonia formed on cultures from class *B*. Likewise the same relative number of antheridia formed on cultures of class *A* and *B* are approximately equal, thus there is an equal chance for reciprocal fertilization.

It has previously been pointed out that cultures of class *A* and *B* are each hermaphroditic; but there is also evidence that within the nuclei of the male and female organs on the mycelia from each class are the genetical qualities of bisexuality. Previous investigators in various fields have demonstrated that male and female structures contain such qualities.

That male and female organs carry bisexual genetical factors has been clearly demonstrated in *Vaucheria hamata* (Vauch.) D.C., by Wettstein (**7**); who demonstrated that the sexual organs of this plant have the ability each to regenerate and form a bisexual plant. He has shown that the contents of an antheridium may form a thallus which is bisexual and in every way similar to the original plant, and in the same way an oogonium may regenerate a bisexual plant.

In the fungi, Couch (**3**) has germinated an egg from an oogonium of Dictyuchus and the thallus from this germinated egg showed that the antheridial nature, although normally latent, was present nevertheless and could be brought out under certain conditions.

It has not been possible, as yet, to germinate the spermatia of *Pleurage anserina* nor to regenerate a mycelium from an ascogonium and thereby demonstrate their bisexual condition directly, in a way similar to that described for *Vaucheria* by Wettstein and for *Dictyuchus* by Couch.

The bisexual qualities of the nuclei in the spermatia and ascogonia can be assumed from experiments showing that after fertilization there is, at reduction division in the asci, a segregation of compatibility factors such that four nuclei are of one compatibility and four of a complementary compatibility; but there is no segregation of sex, all haploid nuclei are genetically bisexual. This is brought out clearly when haploid uninucleate ascospores are germinated and their mycelia produce both male and female

organs. Mycelial cultures derived from haploid uninucleate ascospores fall into two groups which have been designated as belonging to class *A* and class *B* which differ, not in sex, but in respect to compatibility. If we represent this difference of compatibility of class *A* and *B* by two factors S_1 and S_2 respectively we may assume from the experimental evidence that factor S_1 and S_2 are allelomorphic and that associated with each are sex factors. Thus, for example, a haploid uninucleate spore belonging to class *B* carrying factor S_2 gives rise to a mycelium in which all the nuclei carry factor S_2, consequently at the period of maturity during which male and female organs are formed, it seems to be a matter of equal possibility for production of male or female organs, caused by non-gene influence, that determines whether a cell of the mycelium will develop either male or female organs. Thus in cultures of class *B*, derived from haploid uninucleate spores, all the male and female elements contain in each of their nuclei the genetical factor S_2 and the genetical qualities of bisexuality. Likewise in the cultures of class *A*, derived from haploid uninucleate ascospores, all the male and female elements contain in each of their nuclei the genetical factor S_1 and the genetical qualities of bisexuality.

When an ascogonium of class *A* whose nuclei contain factor S_1 is fertilized by a spermatium nucleus containing factor S_2 it is found, judging from experimental results, that from the zygote S_1 S_2 from this cross a segregation within the ascus takes place distributing the factors so that four of the haploid nuclei contain compatibility factor S_1 and the remaining four contain compatibility factor S_2. The mycelium from haploid uninucleate spores, isolated from this cross, produce both male and female sex organs. It is inferred therefore that the spermatial nuclei contain the genetical qualities of bisexuality, and that the female nuclei in the ascogonia contain the genetical qualities of bisexuality. Thus even though the sex organs of each class are morphologically and functionally distinct at the reproductive phase of the life cycle, nevertheless the nuclei of each comprise the potentialities of both sexes.

SUMMARY

1. In *Pleurage anserina* the mycelia derived from uninucleate ascospores are hermaphroditic, self-sterile but capable of cross-fertilization.

2. All of the cultures from uninucleate ascospores fall into two classes, *A* and *B*, on the basis of compatibility; all cultures from single uninucleate spores belonging to class *A* are self-sterile and inter-sterile; all cultures from single uninucleate spores of class *B* are self-sterile and inter-sterile, but any one or all cultures derived from uninucleate spores of class *A* are reciprocally fertile with any one or all cultures derived from single uninucleate spores of class *B*.

3. The cultures derived from the normal binucleate ascospores appear to be homothallic, *i.e.* hermaphroditic and self-fertile, as cultures from single ascospores readily develop perithecia; however, a study based upon hyphal tips shows that the individual nuclei differ from each other, not in sex but in compatibility, each nucleus retaining its individuality so that mycelia from a hyphal tip containing nuclei of the same compatibility class will give rise to both male and female organs, but will prove self-sterile. The mycelia arising from a normal binucleate ascospore therefore, although appearing to be homothallic, that is hermaphroditic and self-fertile, in reality comprise two hermaphroditic cultures growing intermingled as one, each giving rise to both male and female organs which are self-sterile but which are reciprocally fertile.

4. It has previously been pointed out that cultures of class *A* and *B* are each hermaphroditic; but there is also evidence that within each nucleus contained in the male and female organs of each class are the genetical qualities of bisexuality.

5. If we represent the difference of compatibility between class *A* and *B* by two factors S_1 and S_2 respectively we may assume, from experimental evidence, that factor S_1 and S_2 are allelomorphic and that associated with each are sex factors.

6. The sexuality of the giant multinucleate ascospores is essentially like that described for the normal binucleate ascospores, except that there are more initial nuclei concerned; these nuclei belong to two classes, each of which gives rise to both male and female organs which are self-sterile but reciprocally fertile.

7. Because it has been found that cultures from uninucleate ascospores of *P. anserina* are bisexual self-sterile and cross-fertile, it is no longer justifiable to conclude that a culture from a single spore is unisexual simply because it does not produce fruiting bodies when grown alone, or because it requires the mating of complementary mycelia; also it is no longer justifiable to conclude that a culture derived from a single spore is simply bisexual and self-fertile because it does produce fruiting bodies when grown by itself.

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CYTOLOGICAL STUDIES IN THE TREMELLACEAE
I. TREMELLA

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(with plates 47–49 and 11 text figures)

INTRODUCTION

Only one of the comparatively few cytological studies in the Basidiomycetes has been a comprehensive treatment of the Tremellaceae; and even this one, by Neuhoff (1924), (**14**), dealt mostly with the reproductive cells. Considering the position of the group, recognized as low in the Basidiomycete line, it would seem worthy of an intensive study. The present paper records a part of the observations resulting from a study as complete as possible of the members of the group found in New England.

HISTORICAL BACKGROUND

From the time when Tulasne (**17**), in 1853, commented on the absence of "spermatia" (= conidia) in certain species of *Tremella* to that when Brefeld (**2**), in 1888, concluded his long continued cultural studies in the Protobasidiomycetes, little was done other than taxonomically. Brefeld concluded that form, size, variations in color, etc., were of no classificatory value, but that the shape of the conidia, meaning secondary spores, or sporidia, was of prime importance; this enabled him to separate *Exidia,* with its sickle-shaped spores, from *Tremella,* with its spherical spores, but forced him to establish *Ulocolla* as a new genus to care for *Exidia saccharina* with its straight cylindrical conidia. Möller (**13**) even more emphatically asserted the uselessness of external appearances in describing Protobasidiomycetes, and stated that the shape of the hymenium, the form of the basidia, the sterigmata and the

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spores were all essential, as well as the details of spore germination.

The first real cytological study in the Tremellaceae is that of Istvánffi (**8**), in 1895, who followed the external changes in spore outline during development and also the formation of the basidium, which he considered uninucleate when young, through to the completion of sterigmata formation in *Tremella lutescens*. At about the same time, in 1895, Dangeard (**4**) followed accurately the movements and changes in shape of the nuclei from their fusion in the basidium through their migration into the spore, and in *Tremella mesenterica*, stated that the nucleolus became separated from its nucleus during its entrance into the spore. He also described the changes in shape of the fruit-body as its size increased. In 1902, Maire (**11**), in a comprehensive survey of the whole range of Basidiomycetes and with a very complete bibliography, described the chromosomes as being formed from the fusion of many small discrete chromatin granules which he called protochromosomes. He noted that in *T. mesenterica* the conidia which Dangeard (**5**) found to be ultimately uninucleate, were always binucleate as were the secondary conidia budded off from them.

Much more recently, in 1924, Neuhoff (**14**), after a careful comprehensive study of the Auriculariaceae and Tremellaceae, described the basidium as composed of two parts, the basal inflated part in which nuclear fusion and reduction divisions occurred being the hypobasidium from which two to four elongated apical outgrowths, called epibasidia, developed and which ended in slender tubes, the true sterigmata, an interpretation of the basidium accepted by most subsequent students of the Protobasidiomycetes. Neuhoff concentrated his studies mostly on the development of the basidium, but did go into other structures more intensively than had any previous workers. He had only moderate success in *Exidia*, being for the most part unable to follow the stages of nuclear activity. In the case of conidia, he found that "Erst durch die zytologischen Untersuchungen ist ein wesentlicher Unterschied aufgedeckt worden: die am einkernigen Mycel oder an den Sporen erzeugten Konidien sind einkernig (cf. *Exidia glandulosa, E. truncata,* and *E. saccharina*) die in d. Fruchtkörpern gebildeten Konidien besitzen zwei Kerne (*Tremella foliacea, T. lutescens*)." Neuhoff did not follow the development of clamp

connections, which he found to be common only in *Tremella indecorata*.

Shortly after Neuhoff's paper appeared, Kühner (**9**), in 1926, briefly noted the results of a study of *Tremella gemmata* Lév., *Sebacina gloeocystidiata* Kuh., and *Protohydnum lividum* Bres. In *Tremella* he doubtfully stated two chromosomes to be present in the basidium, while his rather poor figures indicate three: he gave no description of nuclear activity in this genus nor in *Sebacina*. In the latter genus he found centrosomes definitely present and also noted in the gloeocystidium the existence of spiral bands which he considered to be vestiges of cytoplasm.

From this brief enumeration of papers dealing with the cytology of the Tremellaceae, it would seem that an intensive cytological study of as many members of the family as possible would be in order and would do much towards furnishing a better basis for connecting this family with the other groups of fungi.

MATERIAL AND METHODS.

That the results be as near normal as possible, the material used in the present study was gathered and fixed in the field during periods of heavy rain, a fact in itself desirable in that it rendered more noticeable fruit-bodies usually overlooked in a dry condition, and also eliminated to a great degree the presence of Fungi Imperfecti so commonly found in the "jelly" of the Tremellaceae.

In the early period of this study, two questions arose: was there any particular time when nuclear activity in the basidium was at a peak, and were there any particular changes to be noted when desiccation occurred? To answer these, several collections were carried on at hour intervals through a 24-hour interval both in rainy weather and during drying. It was discovered that the only factor affecting vital activity was the length of time of exposure to wetting. Drying produced very evident thick walled hyphae forming a protective layer over the surface of the fruit-bodies of *Exidia*, but almost no changes in the other genera studied.

After numerous preliminary fixings in Flemming's Weak Solution, in a picro-mercuric chlorid-alcohol solution,[2] in a chrom-acetic

[2] Picric acid, sat. sol. in 50 per cent alcohol ..... 100 c.c.
Glacial acetic acid ..... 7 c.c.
Mercuric chlorid ..... 5 c.c.

mixture,[3] in Bouin's solution, in Gilson's solution, in Navashin's solution, and others, the second and third solutions were used entirely. Parts of every collection were fixed in each of the chosen solutions.

Thoroughly washed fixed material was embedded part in celloidin and part in paraffin, after very slow dehydration through ten grades of alcohol. Celloidin sections gave greater ease in orienting material and permitted cutting substratum as well as fungus; paraffin sections were thinner. Except in details of the fusion nucleus, where sections were 4 μ, all sections were cut from 8–10 μ thick.

Haidenhain's iron-alum-haematoxylin gave the most satisfactory results, except in those cases where extranuclear material stained almost exactly like the nucleus. As contrast stains, erythrosin, fast green, methyl blue, and safranin gave approximately equally satisfactory results, if allowed to stain only lightly. Delafield's haematoxylin was also used. To overcome the difficulty mentioned above, other stain combinations such as Flemming's Triple, Mallory's Triple, etc., were tried, but with no particular advantage over Haidenhain's. Cajal-Brožek's method, as described by Hruby (**7**), did, however, prove excellent as a stain for dividing nuclei; it was found necessary to increase greatly the time for each of the stains used therein. No one stain proved universally good, but since all material was stained in three or more combinations after each of the two fixing fluids mentioned, reasonably satisfactory results were assured.

TREMELLA

Due to their general habit of growing singly or in small isolated groups, and, in New England, at least, seeming to reach maximum development in cool or even cold weather, members of the genus *Tremella* have not been collected as abundantly as have the other genera used in the present study. All species here studied were found on dead twigs and branches of various deciduous-leaved trees. Identification, especially of young material, was often a matter of considerable difficulty and resulted in the rejection of

[3] Chromic acid ..... 7 gr.
Glacial acetic acid ..... 7.5 c.c.
Distilled water ..... 1000 c.c.

several collections in which the identity was doubtful. All identifications are based on the descriptions of Bourdot and Galzin (**1**), supplemented by references to many other descriptions. The species studied are *Tremella mesenterica* Retz., found abundantly (several collections of *Tremella lutescens* Pers., here considered identical with *T. mesenterica*, since in all collections determined as such, cytological details were identical in every particular, so bearing out the statements of Neuhoff (**15**), Miss Looney (**10**), and others who doubt their separate identity), *Tremella frondosa* Fries,[4] and *Tremella Grilletii* Boud. (*Tremella glacialis* Bourd. & Galz. here is considered identical with *T. Grilletii*.)

Habit

The youngest fruit-bodies of *Tremella mesenterica* Retz. seen had a uniformly smooth surface which became irregularly lobed and folded with increase in size. Except for the pale translucent grey color of the very youngest bodies, all were of some shade of yellow, commonly near Baryta Yellow[5]; the consistency was a firm gelatinous to a soft almost slimy condition when thoroughly wetted. Young fructifications of *T. frondosa* were Light Seal-Brown in color and made up of a single rather firm lobe; old fruit-

[4] The specific identity of this material has been a problem. In color, size, and shape of fruit-body, all recognized as of doubtful value in determining species of *Tremella*, these would be considered *T. frondosa*. Measurements of various parts are equally doubtful, as the following table shows:

Authority	Basidia	Spore	Epibasidia	Hyphae	Conidia
The writer	8×6 μ	5.2×3.5 μ	21×1–2 μ	1.2–2.5 μ	2.5×2 μ
Bourdot & Galz. (1)	14–18–24×11–12–18 μ	7.5–10×7–9 μ	30–45×2–6 μ	1–2–6 μ	3–4.5×2–3 μ
Neuhoff (15)	16–20(–24)×12–18 μ	7–10×7–9 μ			3–4.5×2.5–3 μ
Martin & Huber (12)	7½–14.8×8–16 μ	7–9 μ diam.			
Saccardo (16)	15 μ diam.	5×7 μ			

Several workers, as Bourdot and Galzin (**1**), Coker (**3**), and Miss Looney (**10**) have doubted the separate identity of *T. frondosa*. Others, as Gilbert (**6**), consider it as a good species; the writer's specimens resemble very closely Gilbert's photographs and description. In view of the lack of agreement of descriptions it is to be questioned whether there may not be two species confused under the name *T. frondosa*. Certainly the specimens studied here are distinct from all others studied.

[5] All capitalized colors are taken from Ridgeway's Color Standards and Nomenclature.

bodies varied from Light Seal-Brown to Dilute Cinnamon-Brown, according to moisture conditions during growth, and were very large, the whole lobed body being 30 cm. long and 7 cm. in diameter across lobes. The changes in *Tremella Grilletii* Boud. are of greater interest. The youngest fruit-bodies studied, collected on rotten Elm in September, are discrete bodies from 25 μ to 0.6 mm. in diameter, nearly colorless and with many mature spores, 8.5 $\times$ 6 μ, present on the upper surface. As time went on, the fruit-bodies expanded over the surface of the substratum until in November, some tended to become confluent, although showing their individuality when sectioned. From mid-January to May, however, confluence was complete, it being possible to peel off masses 2 sq. cm. in extent, sections of which showed absolutely no separation of units except at the very edge of the fruit-body. Because of this change and with a range of spore sizes from 8.5–6.5 $\times$ 6–3.5 μ, the writer is inclined to doubt the validity of separating *T. glacialis* Bourd. & Galz. from *T. Grilletii* Boud.

Internal Structure

The mycelium of the species of *Tremella* studied may be traced well back into the substratum, where it is composed of monotonously uniform binucleated segments from 1–1.8 μ in diameter. (PLATE 47, FIG. 1, A, C; PLATE 48, FIG. 1, D-E.) On emergence to form a fruit-body the hyphae differ somewhat from this mycelium, generally becoming slightly larger, more twisting and exhibiting certain specific differences. In *T. mesenterica,* for example, clamps are uncommon and the nuclei of any one segment quite remote from one another (PLATE 47, FIG. 1, B); in *T. frondosa* clamps are very numerous and the nuclei usually close together near the middle of a segment (PLATE 48, FIG. 1, A, B). In these two species considerable quantities of a densely staining substance accumulate at the ends of the segments or even in the middle. No great quantity of this substance was observed in *T. Grilletii,* where other differences were also noted. In this minute species, the hyphae, on emergence from the substratum either spread out in a radial manner to form a minute fruit-body or formed a much tangled thin mat of short binucleate segments over the surface of the wood (PLATE 49, FIG. 1). No clamp connections were seen in this species.

Hypobasidium

As the fruit-body develops it is noticed that there is a dense accumulation of protoplasm in the hyphal tips which form the surface layer. In these dense tips, the basidium initials, the two small nuceli, exactly like those of the mycelium in size and shape, move together and fuse (PLATE 47, FIG. 2; PLATE 48, FIG. 2; PLATE 49, FIG. 2, 3). Even before this fusion occurs, the hypobasidium starts swelling to a spherical to somewhat fusiform body while the hyphal stalk which it ends becomes more and more vacuolate up to the place at which the basal septum will presently form.

With fusion of the two nuclei come several very definite changes: the minute inconspicuous nucleoli fuse to form a very evident large nucleolus (PLATE 48, FIG. 3); the apparently structureless condition of the primary basidial nuclei is replaced by a very noticeable structure which becomes increasingly evident with increase in size, which is very great, both in the hypobasidium and in the included fusion nucleus, particularly in *T. mesenterica* and *T. Grilletii* (PLATE 47, FIG. 3; PLATE 48, FIG. 3–6; PLATE 49, FIG. 4–9). Increase in size produces certain very characteristic specific differences; in *T. mesenterica*, the fusion nucleus, 5 μ in diameter, occupies the center of the very large sub-spherical to irregular hypobasidium whose dimensions average $15 \times 9.7\ \mu$ (PLATE 47, FIG. 4); in *T. frondosa* the maximum diameter of the fusion nucleus is 2.8 μ while that of the very irregularly shaped hypobasidium averages $8 \times 6\ \mu$ (PLATE 48, FIG. 6, 8); in *T. Grilletii* the fusion nuclei in any single fruit-body are very uniform in rate of development to attain a maximum diameter of 3–3.5 μ in the nearly spherical hypobasidia 5–6 μ in diameter (PLATE 49, FIG. 10, 11). Almost from its first formation the chromatin of the fusion nucleus shows itself organized into what at first seems to be an irregular reticulum, but which as development continues becomes more evidently organized into linear patches scattered about at the surface of the nucleus. With continued increase in size these become definitely eight masses of chromatin which gradually contract to form the eight small spherical to elliptical chromosomes (PLATE 47, FIG. 5, A; PLATE 48, FIG. 9; PLATE 49, FIG. 4, 12–14). Thes prochromosomes have been found in every species of the tremellaceous fungi so far studied. With the contraction

of the chromosomes the nucleolus gradually becomes less distinct, finally disappearing at about the time when the chromosomes are definitely formed; the nucleolus seems always to be located at the surface of the nucleus, but never extruded therefrom. As the eight chromosomes separate in reduction division the nuclear membrane disappears, leaving the two groups of four often closely clumped chromosomes to migrate to opposite sides of the hypobasidium. (PLATE 47, FIG. 5, B; PLATE 48, FIG. 7; PLATE 49, FIG. 14.) No definite spindle has been observed in any nuclear division in *Tremella*. The daughter nuclei, much larger in *T. mesenterica* than in the other two species, become definitely reorganized before the second nuclear division occurs (PLATE 47, FIG. 6, 7;

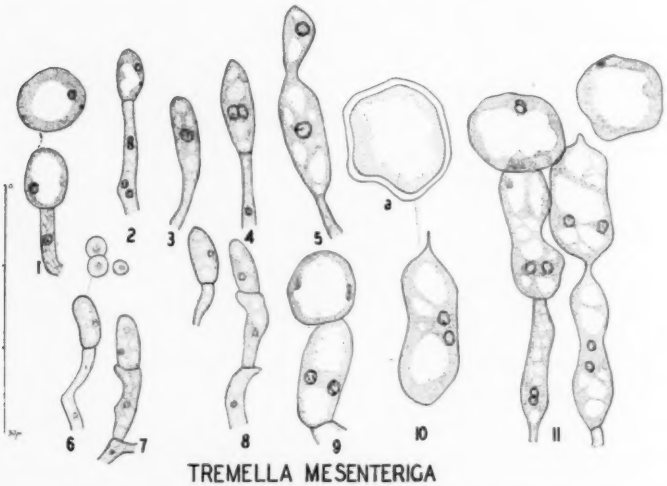

FIG. 1, Uninucleate spherical abortive body; 2, 3, 4, 5, Transitional stages between the uninucleate bodies such as shown in fig. 1 and the binucleate bodies shown in fig. 9; 6, Conidia-like cells embedded in "jelly," with tip of hyphae from which they form; 7, 8, Chains of uninucleate non-spore-like bodies; 9, Large binucleate abnormal "conidia"; 10, 11, Stages in development of abnormal binucleate bodies.

PLATE 48, FIG. 11, 12; PLATE 49, FIG. 15). This second division, with the four chromosomes splitting in halves, occurs either simultaneously (PLATE 48, FIG. 15; PLATE 49, FIG. 16, 18) or one nu-

cleus divides completely before the other starts to divide (PLATE 48, FIG. 14; PLATE 49, FIG. 22). Often the second division fails to take place, particularly in *T. Grilletii*. The four nuclei usually lie at the same level about midway in the hypobasidium (PLATE 47, FIG. 8; PLATE 48, FIG. 13; PLATE 49, FIG. 21), and show a characteristic structure, having a definite small nucleolus and a loosely arranged superficial network of chromatin seeming always to be separable into four groups at all times; this is most easily seen in the relatively large nuclei of *T. mesenterica* (PLATE 47. FIG. 8).

Septum formation has no direct connection with the divisions of the nucleus, indeed, the time of formation of the various septa is extremely variable, but seems rarely to occur until the first division is completed; then the basal and first longitudinal septa rapidly form, the latter apparently basipetally (PLATE 47, FIG. 7; PLATE 48, FIG. 14–16; PLATE 49, FIG. 15–18). The second longitudinal septum is formed immediately after the second division. The planes of formation are extremely variable, although usually nearly central in the hypobasidium.

Epibasidia

Even before the second nuclear division occurs the two or four (rarely three) epibasidia appear as hemispherical bulges filled with a dense accumulation of protoplasm on the apical surface of the hypobasidium. With increasing elongation there appear certain interesting variations which necessitate individual treatment of the three species. In *T. mesenterica* the epibasidia rapidly develop to coarse, slightly twisting structures from 2–3.5 μ in diameter, projecting more or less directly upward from the hypobasidium to the surface of the fruit-body above which they may protrude to a distance of 15 μ, frequently becoming very slightly enlarged (PLATE 47, FIG. 6–12). With development of the epibasidia there occurs a vacuolation of the hypobasidium which progresses from the base of the latter until even the basal portion of the mature epibasidium is included. The development of the epibasidia in *T. frondosa* is even more interesting; here the very slender structures, from 0.8–2 μ in diameter, show definite extremes of development. In the first extreme, which seems to occur mostly at the top of the lobes of the fruit-body, the nearly

straight objects gradually increase in diameter as they push out to the surface of the "jelly" and beyond, and terminate abruptly in the slender sterigmata (PLATE 48, FIG. 17–19). In the second extreme, found mostly at the base of the lobes, the epibasidia continue outward with practically uniform diameter until well beyond the surface of the "jelly" and then expand abruptly to a pronounced one-sided head often $4.8 \times 8\,\mu$, from which the very slender sterigma generally develops laterally (PLATE 48, FIG. 20, 21 C). In *T. Grilletii,* epibasidial development is more uniform: in the younger fruit-bodies mentioned earlier the usually four, but often two, epibasidia are rather chunky objects which just reach to the surface of the "jelly" (PLATE 49, FIG. 19, 20); in the older confluent fruit-bodies, they are longer, more slender structures, generally four to each hypobasidium (PLATE 49, FIG. 22, 24, 25).

As elongation of the epibasidia occurs nuclear migration begins, its first indication being the definite elongation of the nuclei, an elongation which becomes more pronounced as they approach the bases of the epibasidia (PLATE 47, FIG. 10–12). The rate of movement into and through the epibasidia appears to be rather unequal, so that the various nuclei are at different regions in the respective epibasidia (PLATE 47, FIG. 12; PLATE 49, FIG. 25). Subsequent to the passage of the nucleus the protoplasm becomes decidedly vacuolate, the hypobasidium becoming almost empty. As the nucleus passes along the epibasidium there develops from its apical end a slender tube, the sterigma. To pass through this, the nucleus necessarily becomes extremely elongated, the elongation frequently occurring before the nucleus reaches the sterigma (PLATE 48, FIG. 21, C, D).

Spores

Except for the differences in size, the spores of the three species of *Tremella* studied show remarkable similarity in manner of development and germination. The earliest appearance of the spore is a small spherical bubble at the end of the sterigma (PLATE 47, FIG. 12; PLATE 48, FIG. 21, D). This bubble rapidly enlarges to a spherical object with a pronounced apiculus lateral to its attachment to the sterigma (PLATE 47, FIG. 20; PLATE 48, FIG. 21 B, 22; PLATE 49, FIG. 23, 26). When mature the spores of *T. mesen-*

terica average $9 \times 7\ \mu$ and have a pronounced dense apical protoplasmic cap (PLATE 47, FIG. 21); those of *T. frondosa* average $5.2 \times 3.5\ \mu$ (PLATE 48, FIG. 23); while those of *T. Grilletii* are $6 \times 3\ \mu$; all have vacuolate protoplasm, but particularly *T. Grilletii* (PLATE 49, FIG. 27, 28).

On germination, the germ tube (very rarely two form) protrudes, usually laterally (PLATE 47, FIG. 22, 23; PLATE 48, FIG. 24; PLATE 49, FIG. 29–37); at the same time the nucleus starts migrating towards its base, becoming elongated in that direction in doing so (PLATE 47, FIG. 24). Passage through the germ tube into the secondary spore shows exactly the same phenomena as were shown during nuclear migration through the epibasidium, with the primary spore wall collapsing when the nucleus enters the secondary spore (PLATE 47, FIG. 25, 26). This secondary spore is very like the primary spore except in its smaller size (PLATE 47, FIG. 27; PLATE 49, FIG. 36). During germination, no nuclear division occurs. Tertiary spores are apparently also formed at times.

Conidia

Two types of conidia occur in *Tremella*. One of these was seen only in *T. frondosa* and in this species only when spores were germinated in water. From these spores there developed from one to four chains of two to three cells each, budded off from the primary spores. These conidia were always uninucleate; no nuclear divisions were seen in their formation (PLATE 48, FIG. 27).

In *T. mesenterica*, a second type of conidium developed from hyphal tips located among and around groups of hypobasidia. These hyphal tips become many times short septate with each segment usually binucleate. Gradually these segments round off to form spherical binucleate conidia 2.5 to $3\ \mu$ in diameter (PLATE 47, FIG. 16). In many cases the two small nuclei lie one above the other in the plane of vision and are difficult to distinguish; in other cases, particularly in older conidia, the two nuclei seem to fuse, although this blending may be due to approaching disintegration of the contents rather than normal development. Probably the small bodies formed apically on the hyphae of *T. frondosa* (PLATE 48, FIG. 26) are to be interpreted as conidia; these, however, were always found to be uninucleate. Also interpreted as conidia are

the small cylindrical bodies, $5 \times 1.5\ \mu$, found at the ends of slender collapsed tubes in fruit-bodies of *T. Grilletii;* these were binucleate bodies with uniform content of densely staining protoplasm (PLATE 49, FIG. 39).

Abortive structures

Frequently in the fruit-bodies of different species of *Tremella* are found structures perhaps best regarded as abortive, which have been used at various times to explain rather unconvincingly a connection between basidia and conidia, but in any case, of considerable interest. It is difficult to divide these into separate groups on any other basis than that of nuclear condition, some being uninucleate, others binucleate. Through disintegration of the nuclei during development, binucleate bodies become or appear uninucleate; no nuclear fusions ever seem to occur (PLATE 47, FIG. 19; PLATE 48, FIG. 30).

In the binucleate structures the first condition seems always to be a short segmented hypha, in each segment of which are two distinct nuclei (PLATE 47, FIG. 13; TEXT FIG. 2, 4). As development proceeds, the ultimate segment rapidly enlarges, and becomes more and more vacuolate (PLATE 47, FIG. 14; PLATE 48, FIG. 28), until eventually it is a somewhat spherical body having a thin peripheral layer of protoplasm in which are seen the two adjacent disintegrating nuclei (PLATE 47, FIG. 15; PLATE 48, FIG. 29). Eventually this structure becomes a hollow slightly wrinkled empty cell with a conspicuously thick wall, and 10–16 μ in diameter (PLATE 47, FIG. 18). Frequently it becomes separated from the basal portion, often showing a pronounced projection where it was attached (PLATE 47, FIG. 17). Rarely a second spherical cell like the first is formed below it, but more frequently it fails to form at all, or becomes an elongate, curved cylindric, binucleate cell containing a highly vacuolate protoplasm (TEXT FIG. 9, 10). This cell may become a large spindle-shaped cell ($15–20 \times 5–8\ \mu$), which usually ends in a pronounced prong distally and is attached to a similar but more slender segment below (TEXT FIG. 11).

It might be easy to interpret the uninucleate bodies as formed by segmentation of the last described binucleated condition were it not that from their earliest appearance, each segment is obviously

uninucelate. These uninucleate structures apparently fall into two distinct groups; one, seen only in *T. mesenterica* (TEXT FIG. 6, 7, 8) being a short series of segments, of small diameter, from which there seem to form uninucleate conidia-like bodies which at first have very thin uniform protoplasm and eventually become small empty spherical shells embedded in the "jelly." The second uninucleate structure occurs as a chain of large cells which rapidly swell to form spherical to short cylindric segments from 4–8 μ in diameter and often occurring in large much crowded branching groups (TEXT. FIG. 3, 5; PLATE 48, FIG. 33, 36).

It is interesting to note that abortive structures are conspicuously few in *T. Grilletii* (see, however, PLATE 49, FIG. 38), where one encounters but rarely hypobasidia-like structures in which the two small nuclei remain close together without fusing in the highly vacuolate protoplasm (PLATE 49, FIG. 40). Similar structures occur in *T. frondosa* (PLATE 49, FIG. 10, 32). These hypobasidia-like bodies become more and more vacuolate until eventually they collapse and are lost in the developing fruit-body, a fate which is shared by all these structures described as abortive.

DISCUSSION

It is proposed to leave discussion of the possible significance of the various bodies above described until the completion of the present studies. There are, however, certain points which can best be considered at present.

One of these points, namely, the difficulty of separating *Tremella* from *Exidia* has been noted by Brefeld, by Neuhoff, and by others. Brefeld (**2**) held that the form of the conidia (*i.e.*, secondary spores), spherical to "pip"-shaped in *Tremella* and sickle-shaped in *Exidia,* was sufficient for generic distinction. Dangeard decided that the mere presence of true conidia in *Tremella* distinguished this genus from *Exidia.* Neuhoff (**15**) accepted Fries's original separation based on the hymenium, which is unilateral in *Exidia* and amphigenous in *Tremella,* and also used Brefeld's criterion of spore shape in separating the two genera, but pointed out that their exact separation must rest on further research. In his 1931 paper (**15**), he stated that *T. Grilletii* Boud., and *T.*

glacialis Bour. & Galz. are really species of *Exidia* having the sickle-shaped spores which characterize the latter genus. With this statement the writer cannot agree, for all spores seen in the *Tremella Grilletii* here studied are of the typical *Tremella* shape.

Indeed, the writer does not consider that the shape of the primary spores or of the secondary spores developing from them offers the only means for separating the two genera. In the various species of *Tremella* and *Exidia* found in this region, there are two additional points of difference. The first of these is the presence in *Tremella* of the numerous "abortive" structures which are completely lacking in every species of *Exidia* studied. It must be admitted, however, that these bodies are lacking in *Tremella Grilletii,* unless FIG. 40 of PLATE 48 is to be interpreted as such. The second point of difference between the two genera appears when desiccation sets in. In *Tremella* there is then merely a general shrinking together of the parts of the fruit-body with little if any change in any single part. In Exidia, on the contrary, the superficial hyphae of the drying fruit-body become definitely modified to form a dense layer of thick-walled more or less interlacing hyphae which will be described in a future paper.

The second point which should be considered is the noticeably scanty cytological studies of the genus *Tremella.* Istvanffi's study (**8**) of *T. lutescens* and *T. Genistae* needs little comment; he followed mostly the development of the basidium as a whole, noting the formation of the septa whose development he found so easy to follow, but recording little concerning nuclear phenomena save that one nucleus was present in the young basidium and that the spores were uninucleate. Dangeard's more exact study (**4**) of *Tremella mesenterica* Retz was also more a consideration of changes in appearance of the fruit-body, correlated with the formation of conidia followed by basidia, than it was a study of internal development. He did, however, correctly describe the early formation of the fusion nucleus and also the changes occurring when the nucleus passed into the spore.

Considering the relative ease with which specimens may be obtained, it is a surprising thing that, following Dangeard's work, very little attention was paid the genus *Tremella* until Neuhoff's

study. Even though Neuhoff's results are based on a study of insufficient material, they are surprisingly complete.

Nevertheless the work of Neuhoff does leave many gaps which the writer believes his own observations more completely fill because they are based on a large series of specimens, collected throughout the year and particularly in cool to cold weather when they are abundant, and yielded several hundred slides. In the case of the mycelial segments, the writer agrees with Dangeard, Neuhoff, and others that a binucleate condition usually obtains. In these segments, the writer finds that certain evident specific differences appear, as already noted; particularly the relative positions of the two nuclei, which are near together in the center of the segment in *T. frondosa* and *T. Grilletii,* and quite remote from one another in *T. mesenterica.* The writer finds also that clamp connections occur in *Tremella* much more frequently than Neuhoff reported, occurring regularly in both *T. mesenterica* and *T. frondosa.* Moreover, in the hyphal segments of *Tremella,* the presence of a deeply staining substance locally aggregated should also be noted, since this is another means of separating *Tremella* from *Exidia,* where such aggregations are not usually seen.

The development of the hypobasidium (the basidium of the earlier writers) is remarkably constant in all species of *Tremella* and also the related genera *Exidia* and *Sebacina.* Because of this uniformity few points of specific separation other than the size of the mature basidium and its included nuclei occur. The development of the hypobasidium heretofore has not been followed throughout. In the main the various previous studies have resulted only in a series of somewhat unconnected conditions which, for the most part, the writer's studies bear out. That fusion of two small primary basidial nuclei occurs is readily seen; that there is an immediate and considerable increase in the size of the fusion nucleus has also been observed although not specifically noted. In the literature there is but one reference to the details of the fusion nucleus, namely, Dangeard's description of the formation of chromosomes through the fusion of many small bodies, the protochromosomes. The writer is in complete disagreement with this conception. From its first formation the chromatin material of the fusion nucleus is aggregated in masses which become more

and more noticeably organized into definite linear structures, the prochromosomes, whose contraction gradually leads to the formation of the chromosomes. The number of chromosomes is always eight in the diploid condition or four in the haploid.

In the work of previous investigators, studies of the development of the epibasidia (sterigmata of earlier workers) are as scanty as are those of the basidia, and are for the most part directed towards the evaluation of the dimensions of the mature structures and a description of the migration of the nucleus into the spore. That a connection should exist between the nuclei of the hypobasidium and the formation of epibasidia has been argued in other groups of fungi. The present studies definitely demonstrate that such correlation does not exist, showing repeatedly the occurrence of hypobasidia containing two nuclei but having four epibasidia in well advanced stages of development. In other instances an hypobasidium may contain four nuclei but show no sign of formation of epibasidia. The dimensions of the latter seem to depend directly upon the amount of " jelly " present; since " jelly " formation depends on the amount of water absorbed by the fruit-body, obviously atmospheric conditions must have considerable effect on the dimensions of the epibasidia.

Even less variation occurs in the spores which have very definite dimensions in each species here studied. It seems worthy of note, however, that on spore germination no division of the nucleus occurs in species of *Tremella,* the large nucleus migrating into the secondary spore; in *Exidia,* on the contrary, germination of the spore is accompanied by nuclear division, so that many very small nuclei are formed. In the nuclear migration into the secondary spore, as well as in the migration of the nucleus through the epibasidium, the present studies have yielded no evidence that the nucleolus ever becomes separated from its nucleus, nor is there any indication that it is located in any particular region, such as the front of the migrating nucleus. Nor does the extremely elongated shape finally assumed by the nucleus seem imposed upon it merely because of the very narrow diameter of the sterigma; this attenuate condition obtains before the sterigma is reached.

Since so much emphasis has been placed on conidia as a means of separating *Tremella* from *Exidia,* it seemed of value to pay

particular attention to these bodies. The present studies indicate that the succession of basidial formation to conidial postulated by Dangeard does not take place, but rather that the conidia occur in scattered patches among the basidia. The conidia, borne on the hypae of the fruit-body are always binucleate, as Neuhoff has stated. There is evidence, as previously pointed out, that with age there may occur an apparent blending of the nuclei leading to a condition seemingly uninucleate. The conflicting statements regarding nuclear conditions in the conidia, which Dangeard held to be uninucleate and Neuhoff binucleate, may be explained by the uninucleate conidia-like bodies which the writer has described (TEXT FIG. 6). In the main the conidia seem to become engulfed in the increasing mass of "jelly" within which they finally disappear.

On the basis of present study, the writer is convinced that little of value either morphologically or cytologically may be expected from a study of a small number of fruit-bodies, but rather that a large number of specimens carefully collected and prepared is essential in studying the minute structures of the genus. While it is to be hoped that the present study is a step in the right direction and has filled in many of the existing gaps, yet obviously much more long continued patient study of many species will be necessary before adequate basis for taxonomic and phylogenetic relations will be available.

SUMMARY

Fruit-bodies of *Tremella mesenterica* Retz, *T. frondosa* Fries, and *T. Grilletii* Boud. are composed of binucleated segments. The two nuclei are far apart in *T. mesenterica* and close together in *T. frondosa* and *T. Grilletii*. The hymenium, covering the entire exposed surface of the fruit body, is composed of hypobasidia in which two nuclei fuse. This fusion nucleus shows from its inception a definite organization, its chromatin material being aggregated in linear patches, the prochromosomes. These gradually contract when the fusion nucleus reaches its maximum size, to form the eight small chromosomes found in every species studied. These chromosomes separate in reduction division and reorganize into two small nuclei which may divide simultaneously

or individually. Subsequently, these small nuclei become somewhat elongated as they migrate into the epibasidia extending apically from the hypobasidium out to the surface of the "jelly" or beyond. When the nucleus reaches the end of the epibasidium its elongation becomes quite pronounced. Almost no variation in this process occurs in the different species, nor do the spores differ, except in size. These spores, uninucleate bodies borne at the tip of the sterigmata, germinate by a lateral germ-tube and form a secondary spore quite like the primary spore; in this process no nuclear division occurs.

Exceptionally spores give rise to short chains of uninucleate conidia. Conidia are also formed on hyphal tips in and around the basidia, and are binucleate.

Many other bodies also occur in this region. Some of these are uninucleate and in part resemble conidia; others binucleate. The presence of these "abortive" structures in *Tremella* seems to separate this genus from *Exidia*, which lacks them.

ACKNOWLEDGMENT

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EXPLANATION OF PLATES

PLATE 47

Tremella mesenterica Retz.

In this and all other plates, as well as in text figures, all figures have been drawn with the aid of a Camera lucida, at a magnification of 3800 ×, and subsequently reduced to about 0.3, *i.e.*, to a magnification of about 1150 ×. In addition an absolute scale of dimension is included in all plates.

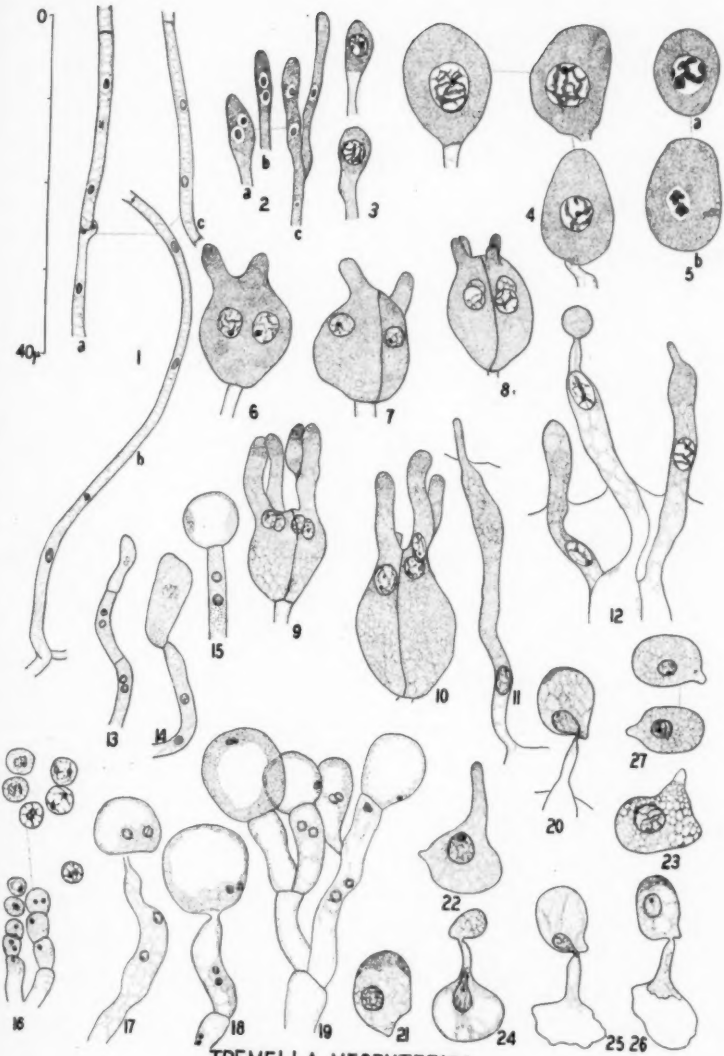
Fig. 1, *a, b, c.* Binucleate segments of hyphae, with oil-like substance accumulated near end-walls and also in the center of the segment in fig. *a;* 2, hybosadium initials, each showing the two nuclei before fusion; 3, hypobasidium showing the enlarging fusion nucleus and the forming basal septum; 4, hypobasidium showing the large mature fusion nucleus and the complete basal septum; 5, *a,* hypobasidium showing the nucleus in which are eight nearly mature chromosomes, *b,* the same with the chromosomes separating, four in each group; 6, hypobasidium showing the two daughter nuclei resulting from the first division and the two relatively thick epibasidia forming apically; 7, the same with the two daughter nuclei separated by the first longitudinal septum; 8, hypobasidium showing four basidial nuclei and four apical epibasidia; 9, hypobasidium showing the four nuclei migrating toward the bases of the half-developed epibasidia; 10, the same showing the slightly elongated nuclei entering the bases of the epibasidia; 11, epibasidium showing the elongated nucleus migrating toward the slender sterigma which projects above the surface of the "jelly"; 12, epibasidia showing the vacuolate protoplasm following the passage of the elongated nuclei. Sterigma of the central epibasidium bearing a half-formed spore; 13, binucleate segments of tip of hypha; 14, 15, stages showing the enlargement of the apical segment of a hyphal tip such as fig. 13; 16, binucleate conidia breaking loose from tip of chain. Upper two conidia to left showing vague blending of nuclei suggestive of fusion; 17, 18, chains of segments similar to those in fig. 14, but showing the penultimate segment becoming elongated and vacuolate; 19, branching group of binucleate cells similar to those

show in figs. 17, 18; 20, nearly mature spore showing the nucleus just leaving the sterigma and the apical "cap" of protoplasm beginning to form; 21, mature spore showing dense apical "cap" and somewhat vacuolate protoplasm; 22, 23, spore germinating by lateral germ tube; 24, germinating spore showing the elongating nucleus leaving the extremely vacuolate protoplasm of the spore to enter the germ tube at the tip of which a secondary spore is forming; 25, secondary spore showing the nucleus just entering; 26, nearly mature secondary spore at the tip of the germ tube; 27, mature secondary spores.

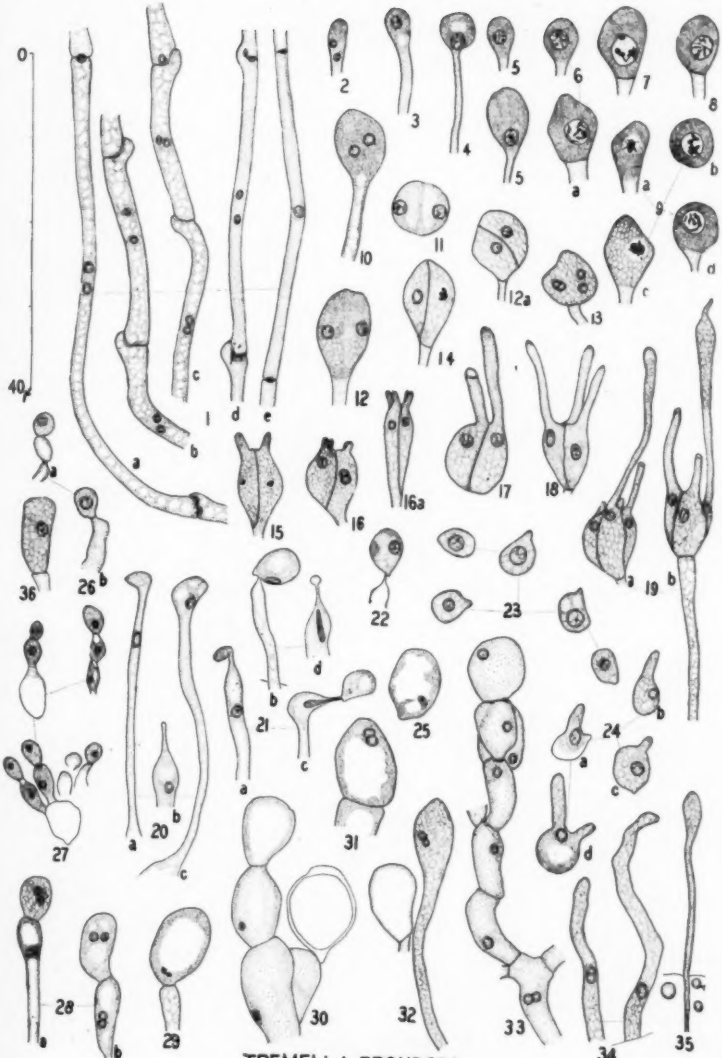
PLATE 48

Tremella frondosa Fries.

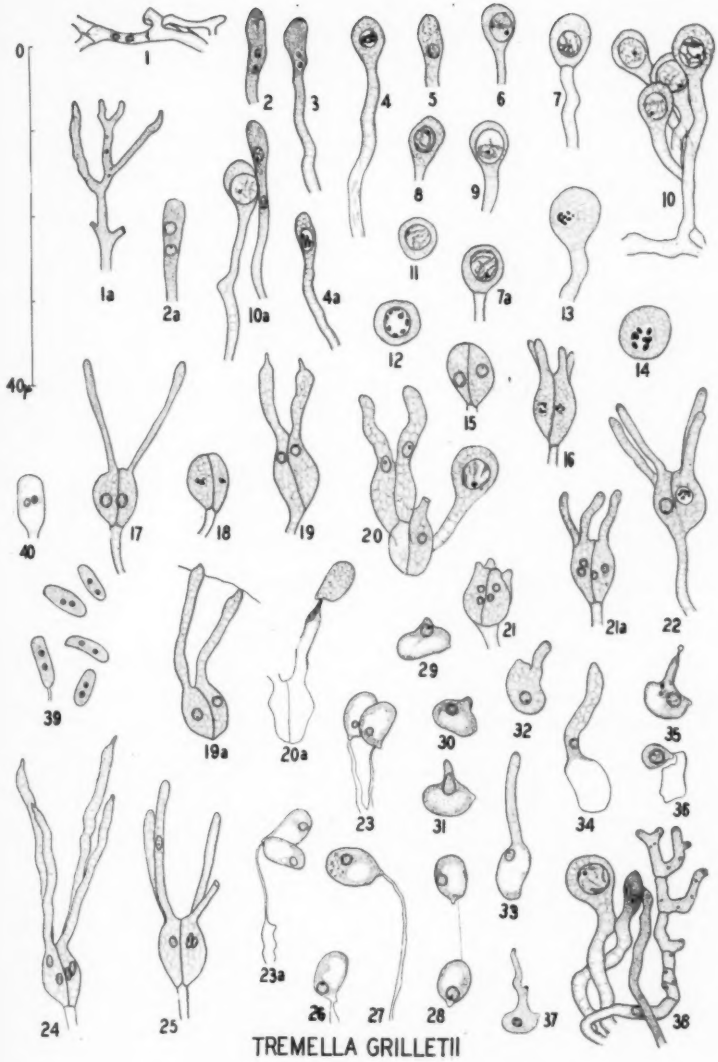
Fig. 1, hyphal segments: *a, b,* binucleate type found in fruit-body; *c,* the same beneath the hymenium showing the various arrangements of the two nuclei; *d,* binucleate segments in substratum; and e, uninucleate segment in substratum; 2, young hypobasidium with primary basidial nuclei before fusion; 3, same, showing the fusion nucleus before the nucleoli fuse; 4–6, hypobasidia showing enlarging fusion nucleus; 7, hypobasidium showing eight chromosomes in two equal groups; 8, same, showing mature fusion nucleus and the basal septum; 9, same, showing: *a, c,* the closely clumped chromosomes, and *b, d,* the diakinesis of the first division; 10, basidium-like binucleate hyphal tip; 11, cross section of hypobasidium showing two nuclei near the walls; 12, the same in side view; 12*a*, the same showing an oblique longitudinal septum separating the two nuclei; 13, hypobasidium showing four distinct nuclei; 14, same, showing one of the two nuclei dividing; 15, same, showing both nuclei dividing simultaneously and epibasidia forming apically; 16, hypobasidium showing four epibasidia forming at its apex. Basal septum as yet unformed; 16*a*, unusually elongate hypobasidium; 17, hypobasidium showing two well-developed apical epibasidia; 18, same, showing three well-developed apical epibasidia; 19, same, showing four epibasidia to the bases of which the nuceli are migrating; 20, epibasidia showing the nuclei near their swollen apices; 21, same, ending in slender sterigma directed either vertically (*d*) or laterally (*c*), at the tip of which the spore is forming. Nucleus either in the epibasidium (*a*) or very much elongated in the swollen epibasidial tip prior to passage through the slender sterigma (*c, d*), or in the spore close to the wall (*b*); 22, epibasidial apex showing the nearly mature spore at its tip; 23, mature uninucleate spores; 24, spores germinating by single lateral (*a, c*) or terminal (*b*) germ-tube, or (*d*) with two germ tubes; 25, spore-like body cut off from hyphal tip similar to that of fig. 10; 26, 27, uninucleate conidia cut off from hyphal tips, (26) or budding from spore (27); 28, binucleate cells in short chains at surface of fruit-body; 29, 31, stages in the formation of the spore-like body shown in fig. 25; 30, swollen vacuolate cells formed in chains and showing progressive nuclear disintegration. Thick-walled empty cell at right; 32, basidium-like hyphal tip with one of surrounding vacuolate segments, such as fig. 28; 33, branching chain of uninucleate segments generally found around the hymenium; 34, 35, erect hyphal tips which project rigidly above the surface of the fruit-body; 36, truncate uninucleate body sometimes occurring at edge of hymenium.



TREMELLA MESENTERICA



TREMELLA FRONDOSA



TREMELLA GRILLETII

PLATE 49

Tremella Grilletii Boud.

Fig. 1, short binucleate segment of mycelium; 1*a*, branching tip of hypha in very young fruit-body; 2, 2*a*, 3, young hypobasidia showing two nuclei; 4, hypobasidium showing the nucleus in which the eight chromosomes are distinctly present; 5, hypobasidium showing fusion nucleus before the nucleoli fuse; 6, same, showing enlarging fusion nucleus; 7, same, showing the prochromosomes definitely present in the fusion nucleus; basal septum distinct; 8, 9, 10, nearly mature hypobasidia; 10*a*, mature hypobasidium together with a young hypobasidium into which the enlarging nuclei are just migrating; 11, transverse section of the mature hypobasidium; 12, the same showing the peripheral prochromosomes in section; 13, hypobasidium showing the eight distinct chromosomes; 14, transverse section of slightly later condition than fig. 13; 15, hypobasidium showing the two daughter nuclei separated by the first longitudinal septum; 16, hypobasidium in which the two nuclei are dividing simultaneously while four epibasidia are forming apically; 17, hypobasidium showing two nearly mature epibasidia; 18, hypobasidium showing the two nuclei dividing simultaneously before any epibasidia appear; 19, 19*a*, hypobasidia showing two mature epibasidia; 20, group of hypobasidia showing (left coarse epibasidia; 20*a*, hypobasidium collapsing as the elongated nucleus passes into the well advanced spore; 21, 22, 24, hypobasidia showing the development of the four apical epibasidia; 23, vacuolate mature spores at tip of collapsed epibasidia; 25, hypobasidium showing one of the nuclei migrating; 26, nearly mature spore showing large central vacuole and vacuolate condition of apiculus and supporting sterigma; 27, abnormally large spore formed at tip of collapsed sterigma; 28, mature spores showing vacuolate condition and distinct apiculus; 29–33, stages in the development of the germ tube; 34, spore similar to that of fig. 27, showing rather thick, apical germ tube into which the nucleus has just migrated; 35, mature spore showing germ tube at the tip of which the slender sterigma is formed to bear the spore; 36, mature secondary spore still attached to collapsed primary spore; 37, spore germinating by a branching germ tube; 38, structures found in young fruit-body, in hypobasidium containing large fusion nucleus (left) young hypobasidium before nuclear fusion (center). Paraphysis and septate branched hypha (right); 39, binucleate conidia; 40, truncate hyphal tip sometimes found among the basidia.

NORTH AMERICAN HYPHOMYCETES II. NEW SPECIES AND A NEW GENUS

David H. Linder

(with plate 50)

Among the specimens of Fungi Imperfecti that the writer has recently had occasion to study, three stand out as quite distinct from any that have been described.

The first of these, communicated by Professor H. S. Jackson of the University of Toronto, grew on the white resupinate fructification of either *Corticium* or *Peniophora,* where it formed a dark brown and somewhat cottony layer. Microscopically the form is unique among the phragmosporous forms of the Dematiaceae. The type of branching of the conidiophores is strongly reminiscent of that seen in the genus *Botrytis,* while the method of spore formation is so different from anything found in near-by genera that it is somewhat difficult to place the form in the proper tribe. It approaches *Spondylocladium* most closely since the spores occur in whorls around the upper part of the terminal and subterminal cells of the main axis and branches, but it differs from that genus since, instead of producing the conidia in whorls and directly upon the simple main axis, the conidia are produced singly on short, simple, one-celled branches that are formed verticillately (plate 50, fig. 1, 3). It is this arrangement that suggests that the species should be placed in the form-tribe Acrothecieae, yet in this group it is somewhat anomalous since the genera of this tribe are characterized, in addition to the verticillate arrangement of the conidia, by the simple erect conidiophores, whereas the species under discussion is ramose, and the conidia are not attached directly to the main axis of the conidiophore. Therefore, because of the striking characters that this fungus presents, a new genus and species is proposed.

[1] Contribution from the Laboratories of Cryptogamic Botany of Harvard University, No. 129.

Spondylocladiella gen. nov. PLATE 50, FIG. 1–3

Fungus conidiophoris rectis vel adscendentibus, ramosis et sursum ramulos simplices verticillate vel subverticillate gerentibus; conidiis in ramulis acrogenis, unicis, fuscis, ellipsoideis, 2-(raro 1- vel 3-) septatis.

Condiophores erect or ascending, branched and bearing verticillately or subverticillately on the upper part of terminal or subterminal cells, short, one-celled branchlets; the conidia solitary and acrogenous on the branchlets, fuscous, ellipsoid, 2- (rarely 1- or 3-) septate.

Spondylocladiella botrytioides sp. nov.

Coloniae effusae, breve bombycinae, atro-fuscae; conidiophoris 300–400 × 5–9 μ, rectis vel adscendentibus, septatis, infra hyalinis vel subhyalinis, sursum fuscis pellucidisque, ramosis, ramis plerumque unilateralibus nonnumquam alternis, cellulis terminalibus vel subterminalibus ramulos unicellulares verticillate vel subverticillate gerentibus; ramulis subhyalinis vel dilute fuscis, 8–12 μ longis, 4–7 μ diametro; conidiis in ramulis solitariis, acrogenis, sessilibus, ellipsoideis vel inaequilateraliter ellipsoideis, fuscis, 12–20 × 7.5–9 μ, (1)—2-(3-)septatis, leniter ad septa constrictis.

Colonies effuse, short-cottony, dark brown. The conidiophores 300–400 × 5–9 μ, erect or ascending, branching above, septate, the lower cells hyaline or subhyaline, the upper cells light fuscous and pellucid, the branches mostly unilateral or occasionally alternate; the terminal or subterminal cells bearing 1-celled branchlets verticillately or subverticillately; the branchlets subhyaline or dilute fuscous, 8–12 μ long, 4–7 μ in diameter. The conidia solitary, acrogenous, sessile, on the 1-celled branchlets, 12–20 × 7.5–9 μ, (1-) —2- (3-) septate, slightly constricted at the septa, ellipsoid or inequilaterally ellipsoid, fuscous and more deeply colored than the conidiophore.

Growing on the hymenial surface of *Corticium* (?). Toronto, Canada, December 26, 1931. H. S. Jackson, *3199*, type in Farlow Herbarium and the Herbarium of the University of Toronto.

The second species to be described is a very pretty one which was found in the greenhouse growing on flower pots made from tobacco stems. This species, growing in association with *Oedocephalum* sp. and occasionally on the ascocarps of *Ascobolus* sp., formed no conspicuous colonies and was evident only as scattered conidiophores or small tufts. A microscopic examination showed that it belonged in the genus *Dactylaria*, near *D. Orchidis* and *D. candida*, but it differed from these in the size and the number of septations of the conidia. To indicate its relationships, a key

has been compiled from the descriptions of the known species as given in the various volumes of Saccardo's Sylloge Fungorum. With the exception of *D. simplex* (Pr.) Sacc. and *D. graminum* (Schw.) Sacc. for which no measurements are available, all species are to be found in the key which follows:

1. Conidia over 30 μ long ..... 2.
1. Conidia less than 30 μ long ..... 6.
2. Conidia less than 12 μ diameter ..... 3.
2. Conidia more than 12 μ in diameter ..... 5.
3. Conidia 4-guttulate, then 3-septate, the conidiophores aurantiaceous *D. Orchidis* Cke. & Mass.
3. Conidia 4–8-septate ..... 4.
4. Conidia 4–5 septate, 45–56 $\times$ 7–9.5 μ *D. candida* (Nees) Sacc.
4. Conidia 5–6–(8)-septate, 27–45 $\times$ 8–11 μ *D. pulchra* Linder.
5. Conidia 5–6-septate, 45 $\times$ 15 μ *D. oogena* (Mont.) Sacc.
5. Conidia 6–7-septate *D. oogena* var. *Pancierii* Sacc.
6. Conidia 18–22 $\times$ 7–9 μ *D. parasitans* Cav.
6. Conidia less than 7 μ in diameter ..... 7.
7. Conidia 8–10 $\times$ 2.5–3 μ *D. mucronulata* Ellis & Langl.
7. Conidia more than 12 μ in length ..... 8.
8. Condia 20–25 $\times$ 4 μ; apex of conidiophore often spatulate inflated *D. purpurella* Sacc.
8. Conidia 16–26 $\times$ 4–5 μ; apex of conidiophore not inflated *D. echinocephala* Massal.

It can be seen from the above key to species that this one is quite distinct and hence the name *Dactylaria pulchra* is proposed.

Dactylaria pulchra sp. nov. PLATE 50, FIGS. 4–5

Hyphis sterilibus in substrato immersis; conidiophoris erectis, 1–3-septatis, 100–175 $\times$ 3.5–4 μ leniter fastigatis, hyalinis, simplicibus vel ad apices breve ramosis; et sterigmates terminales et laterales gerentibus conidiis (27)–34–40(45) $\times$ (7)–9–11 μ, hyaline vel dilute roseis, oblongo-fusoideis, (4–)–5–7–(8–)-septatis.

Sterile hyphae growing in the substratum. The conidiophores erect, one to three-septate, 100–175 $\times$ 3.5–4 μ, slightly tapering upwards to 2–2.5 μ, hyaline, simple or short-branched at the apices and bearing terminal and lateral sterigmata on the somewhat zig-zag terminal or subterminal cells. The conidia (27)–34–40–(45) $\times$ (7)–9–11 μ, hyaline or dilute rose colored in mass, oblong fusoid and occasionally slightly curved, (4–)–5–7–(8–)-septate.

Growing in a greenhouse on flower pots made of tobacco stems. Cambridge, Massachusetts, December 1933, *D. H. Linder*, type, deposited in the Farlow Herbarium, Harvard University.

The third species to be described belongs in the dark-spored group of the dictyosporous Tubercullariaceae. It is characterized by the pulvinate or irregularly hemispherical sporodichia upon which chains of spores are formed in basipetalous succession. The spores, at first dilute fuscous and sparsely warted, are transversely septate, with maturity become conspicuously and rather closely warted, nearly black and opaque, and both transversely and longitudinally septate. These characters place the species in the genus *Bonordeniella,* but since the conidia are irregularly ellipsoid rather than irregularly globose, as is true of *B. memoranda* Penzig & Sacc.,[2] the type and only species hitherto known, it becomes necessary to apply to the American species a new name, and because of the rough warty appearance of the spore, *Bonordeniella aspera* is proposed.

Bonordeniella aspera sp. nov. PLATE 50, FIGS. 6–8

Sporodochia atro-brunnea fere atra, solitaria vel gregaria, interdum coalescentia, irregulariter hemisphaerica vel lobulata, usque 1.5 cm. diam., extus ruguloso-pulveracea; conidiophoris 20–40 × 2–3 μ, infra hyalinis vel subhyalinis superne fuscescentibus; conidiis ellipsoidesi, 19–28 × 7–11 μ, atrofuscis, primum transversa septatis deinde transversa et longitudinaliter septatis, leniter ad septa constrictis, verruculoso-asperatis, in catenulas simplices digestis, catenulae saepe cellulis minoribus hyalinisque interruptae.

Sporodochia dark brown, almost black, solitary or gregarious, occasionally coalescing, irregularly hemispherical or lobulate, up to 1.5 cm. diam. or larger by fusion, externally rugulose-powdery; conidiophores 20–40 × 2–3 μ, hyaline or subhyaline below becoming darker above; conidia ellipsoid, 19–28 × 7–11 μ, deep fuscous, opaque, at first transversely septate becoming transversely and longitudinally septate, slightly constricted at the septa, warty roughened, formed in simple chains, the chains often interrupted by small hyaline cells.

Haverhill, Massachusetts, on well weathered fence-post (probably oak), Oct. 29, 1933, *S. K. Harris.* Type deposited in the Farlow Herbarium, Harvard University.

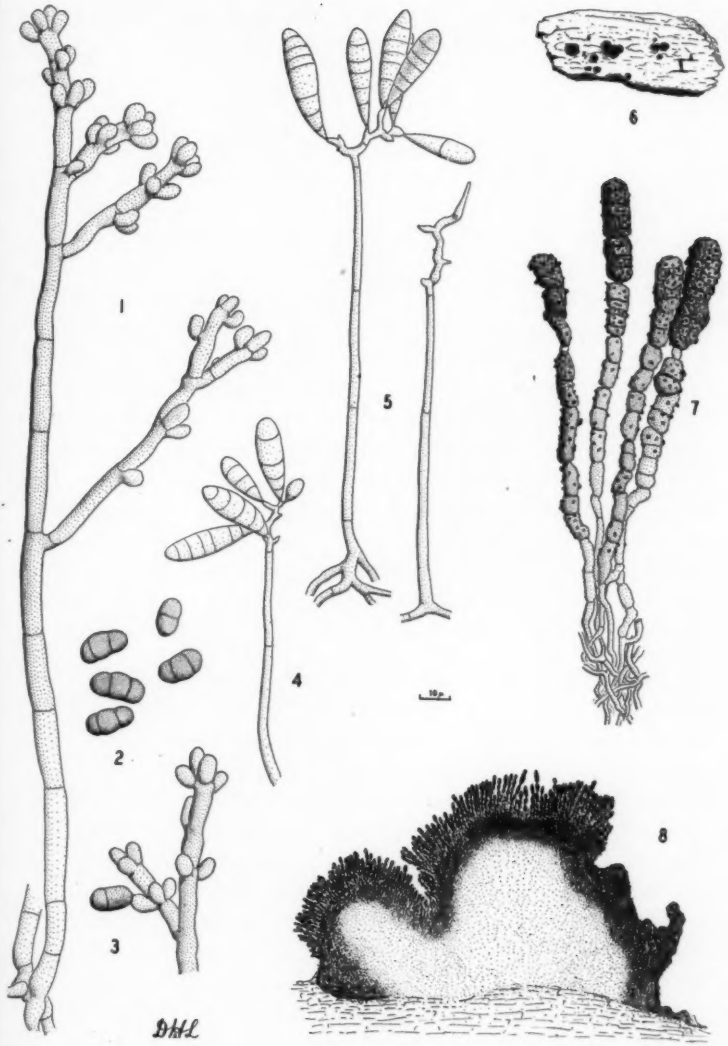
HARVARD UNIVERSITY,
CAMBRIDGE, MASS.

[2] Penzig, O. & P. A. Saccardo, Fungi Javanici p. 116, *pl. 80, fig. 4,* Leiden 1904.

EXPLANATION OF PLATE 50

All drawings except figure 6 were made with the aid of a camera lucida from material mounted in lactophenol and with the exception of figures 6 and 8 are reproduced at a magnification of × 500.

Fig. 1–3, *Spondylocladiella botrytioides* showing the characteristic type of branching of the conidiophores, the verticillate or subverticillate arrangement of the 1-celled sporogenous branches, the attachment of the conidia, and the variations in the shape, size, and septation of the conidia; 4–5, *Dactylaria pulchra* showing the typical conidiophores and manner in which conidia are produced; 6–8, *Bonordeniella aspera,* fig. 6 depicting the habit of the fungus, natural size; 7, shows the conidiophores arising from the stromatic tissues and bearing chains of spores, some of which have reached maturity and are deep brown, opaque and transversely and longitudinally septate. The chains are occasionally interrupted by small hyaline cells; 8, shows a section through the stroma, the context of which is hyaline within, becoming fuscous towards the surface from which arise the conidiophores. × 50.



HYPHOMYCETES

MYCOLOGICAL LETTERS FROM M. A. CURTIS 1856–1861

NEIL E. STEVENS

The herbaria accumulated in this country prior to the war between the States were largely the work of men who were amateurs in the best sense. Even the few who held professorships or curatorships were in constant communication with workers who collected, preserved, and exchanged plant specimens as a pastime and with the zeal now characteristic of stamp collectors. An intimate picture of the manner in which the enthusiasm and energy of the tyros was directed by the more experienced workers is found in the letters of M. A. Curtis to the Rev. Joseph Blake. These letters form a part of Blake's botanical correspondence and are preserved with his herbarium in the University of Maine. For the privilege of examining this interesting collection and of publishing the letters here quoted the writer is indebted to the University of Maine and particularly to Professor F. H. Steinmetz of that institution. I am also indebted to Mr. Arthur H. Norton, Secretary of the Portland Society of Natural History, for the following brief biographical sketch:

"Joseph Blake was born in Otisfield, Maine, January 20 1814, and died at Andover, Massachusetts, May 6, 1888. He was graduated from Bowdoin College in 1835 and Bangor Theological Seminary in 1840. Ordained as Pastor at Cumberland, Maine, in 1841; he served there until 1859. He was Pastor at Gilmanton, New Hampshire, from 1860 to 1878, and resided at Andover, Massachusetts, from 1878 until his death in 1888. He married a lady from Wells, Maine, hence the large collections of plants which he made in that town. He was a very active collector, making a goodly herbarium, largely from the towns of Cumberland and Wells, Maine; and Gilmanton, New Hampshire. His own collection, after his death, was purchased by friends and presented to the Maine State College, now the University of Maine, at Orono. During his life he contributed

generously to various herbaria, including this in Portland. Blake published very little though he was responsible for the list of Graminiales in Goodale's Catalogue of the Plants of Maine published in the Proceedings of the Portland Society of Natural History I, (1) and (2), 1862 and 1869. Mr. Blake's coauthorship appears in 1869 in the second part."

References to Blake's collections are found in the papers of several botanists and accounts[1,2] of his two ascents of Mount Katahdin, taken from manuscripts prepared for the entertainment of his children, were published by Mr. Norton in the Maine Naturalist. On the second trip he discovered *Saxifraga stellaris.*

Curtis' mycological work is too well known to need review and biographical sketches[3] are available.

THE OPENING OF THE CORRESPONDENCE

The correspondence between Curtis and Blake, like many similar ones, began by a sort of "Letter of Introduction" from Asa Gray. Blake after collecting flowering plants for some years had become interested in fungi and received the following reply to an inquiry for sources of further information.

CAMBRIDGE, Oct. 13, 1855.

Dear Sir:

Just home from a hurried & sudden visit to London and Paris, I find your favor of Sept. 8th.

Publications on Fungi—such as you want—there are none for N. Amer. On this subject you should correspond with Charles J. Sprague, Esq., Boston, who has lately taken up the subject, and especially with Rev. Dr. M. A. Curtis, Society Hill, South Carolina. He will name the fungi you send him.

I have *Saxifraga azoides* from Willoughby Mt., but not *S. Aizoon.* Send me a leaf from yr. specimen, and I will tell you if it is the plant—which will be new for New England, & will go in new edition of the Manual.

Excuse great haste

Yours truly,

A. GRAY.

[1] Blake, Joseph. An Excursion to Mount Katahdin. Maine Naturalist **6**: 71–73. 1926.

[2] Blake, Joseph. A Second Excursion to Mount Katahdin. Maine Naturalist **6**: 74–83. 1926.

[3] Shear, C. L. and Stevens, N. E. The Mycological Work of Moses Ashley Curtis. Mycologia **11**: 181–201. 1919. And other papers therein cited.

Those were leisurely days and no doubt the life of a country clergyman in Maine in the middle of the last century held other than botanical activities. It was apparently over a year later that he wrote Curtis and in reply received the following very encouraging letter:

SOCIETY HILL, S. CAR.; Oct. 30th, 1856.

Dear Sir:

Yours of the 17th rec'd this morning, could hardly have reached me at a more unlucky moment than the present. I am very much pressed for time & do not expect much leisure for botanical studies during the remainder of the present year. Nevertheless, to show my good will, & to encourage you in further investigations in the interesting but obscure field of Mycology I have made a hurried examination of your specimens, & report as well as I can under the circumstances on next leaf. I have also selected a few specimens for you, taken (all but one) from letters just rec'd from other regions & now lying before me. These will, so far, help to furnish material for knowledge in this much neglected department of science.

I have no correspondent in Maine, though I occasionally receive specimens from that State, & shall be very glad if you continue to send me specimens of Fungi. I will do what I can towards facilitating your study of the Fungi. You must, however, bear in mind, that, among objects so obscure & so little known, I can not be expected to know everything at sight, & so may not be able to gratify your curiosity in all cases until after long delay. I have had species for many years in the hands of the best European Mycologists, & have not yet rec'd their decisions. Besides, my correspondents are numerous, & it is no easy matter to attend to all. Within a week I have rec'd over 600 specimens of Fungi. Remember too that most of the species have to be subjected to the Microscope. Under such circumstances you will find cause to have patience with me. But I will do my best, & hope I shall be able to satisfy you on the whole.

What books, & what sort of microscope, have you?

I take the liberty of sending a poison mixture, which you will find a sine qua non, if you would preserve your specimens from insects.

	Corrosive sublimate	Z iv
	Sulphuric ether	Z iii
Dissolve & add		
	Alcohol	Z iii
	Spt.s of turpentine	Z ii

The thinner species should be washed with this mixture by means of a feather. The larger fleshy & woody forms, after being dried, should be more thoroughly washed or even saturated within it & then dried again. Otherwise, they will inevitably be destroyed by larvae and insects.

In much haste

Yours respectfully,

M. A. CURTIS

Still another year elapsed and another letter was received from Curtis before the sending of specimens began.

HILLSBOROUGH, N. CAR. Sept. 1st '57

Dear Sir,

Last October I recd a letter from you enclosing specimens of Fungi, and I immediately replied, giving names of the species. I judged from your letter, that you were anxious to prosecute the study of these obscure and much neglected productions, and were desirous of such aid as I can give. But having heard nothing further from you, I have thought that a letter from one or other of us has miscarried, and hence that you may have thought me unwilling to continue the service required. I take the liberty, therefore, of assuring you that I shall be very glad to receive specimens from your State, as it will serve to increase my knowledge of the range of species, and will very likely bring to light some things before unknown. In assisting you, therefore, I shall be benefiting myself, and I shall very cheerfully determine species for you as far as I am able. From Connecticut, Massachusetts, and N. Hampshire, quite a number of new species, and of species before unknown to me as American, were sent to me last year. From Maine I have none but a few from the vicinity of Portland, besides those you have sent. I shall be well pleased if you can take such interest in these things as will induce you to persevere in their collection.

Very respectfully

M. A. CURTIS.

Following the actual sending of specimens there came from Curtis more specific directions for collecting and preserving specimens, a list of the best books on fungi and suggestions for securing and equipping a microscope. The letters covering these points are here published without correction or alteration except the omission of occasional "lists" of identifications. They furnish a picture of the methods of work of this pioneer American Mycologist more detailed in some respects than any hitherto available.

HILLSBOROUGH, N. CAR. Sept. 20, 1857.

Dear Sir.

This morning I recd. yours of the 16th and on last page you have report upon the enclosed species. Two of them are perhaps new. Though you should not make a vigorous study of these things, you may do good service to science by collecting whatever falls in your way. American Mycology is already indebted to you, as you see above, for some additions to her stores. Last year Mr. Sprague of Boston, & Ch. Wright of Conn. added about 50 new species to my stock, from N. England.

As to Books, you need Systema Mycologicum, (3 vols.) Elenchus Fungorum, (1 vol.) & Epicrisis Syst: Mycol: (1 vol.) by Fries. These will cost about $14.00, & can be had through any importing Bookseller of Boston, I presume Bailliére of N. York is most familiar with works of this

kind, & I have always found him prompt, & reasonable in his charges. Having houses in London, Paris, Madrid, &c he has unusual facilities for accommodation.

You will find Payer's Botanique Cryptogamique very serviceable, which is illustrated with beautiful figures. Cost about $3.00.

Berkeley's "Introduction to Cryptogamic Botany" will be useful as an *Introduction*, & has some figures of Fungi, a goodly number of them American species. Price $5.00.

In advising you in regard to a Microscope, I must first know how much you are willing to expend for an Instrument. A suitable Compound Microscope can not be had under $75. If you can spare $100. I should advise one of Robbins' Instruments at that price. But if your means are too small for such an outlay & for such a purpose, as is generally the case with country Clergy, I should recommend one of Chevalier's Doublets. I have never used any thing else, & I can see with it objects measuring only 1/10,000 in. in length. I happen to have a spare one, Mr. Berkeley having presented me with one. If you will run the risk of its transportation by Mail, & give me what it cost ($7.00) I will let you have it, as I have no use for two. I use it in the stand of a small Compound Microscope which cost $10. If you have nothing of the sort, you can have a stand made for it, of which I will give you the idea. The Doublet is about an inch in diameter, & 2 lines thick, & can go by mail easily enough.

Fleshy Fungi are preserved by pressure in paper like Flowering Plants. But I let them become flaccid in the air before subjecting them to a gradual pressure. The paper must be changed frequently at first. It is rather troublesome & specimens are often badly injured by Larvae before they can be dried. They are so much changed by the process, that the essential characters can not always be gathered from dried specimens. It is therefore necessary to write out full descriptions from the fresh specimens. And as the dried specimens are eagerly sought by insects, they must be poisoned as soon as dried, or they will soon be consumed.

[Here follows a list of fungi]

In sending again, do not repeat your Numbers, but continue on from 14. Otherwise future references, (which will be necessary in case of species not at once determinable) will not be intelligible.

Always give the name of the object on which the Fungus is,—the specific name when possible.

The important notes to be made of the fleshy Fungi are the *dimensions* of Cap & Stipe:—Color of the different parts:—whether the Gills are *free* from the Stipe or attached to it:—if the Stipe be solid, hollow or fistulous,—*color* & *taste* of the juice, if any;—& *odor* of the plant, when describable.—A very essential character is the *color* of the *Spores*. These are procured by laying the specimens (gills or pores downward) for a few hours upon white or blue paper. These Spores should be sent with the specimens to be glued with them upon the tickets. The fuller your descriptions the better. It is quite impossible to determine species often times without them;—& with them, such is the number of these things, it is often difficult. It is only when we are pretty familiar with a species, that we can determine it without notes. If you could also give full drawings of the specimens, it would be a great help. Sprague sends me very fine sketches.

My poison mixture, which I have found very effectual, is composed of

Corrosive Sublimate—Z IV
Sulphuric Ether —Z III
Dissolve & add
Alcohol —Z III
Spts. Turpentine —Z II

On thin specimens I generally apply it with a feather. Upon large & especially the woody species I usually pour it so as to saturate at least all the surface. These liquids are so volatile, that specimens dry very quickly after the application, & may be put almost immediately in the Herbarium. Bulky forms are of course put on shelves in a Cabinet.

Unless you have a pretty large collection, say 100–300 of bulky specimens, it is hardly worth while to send me a package at present. By next Fall, you may perhaps have enough, when I will give directions for forwarding; or in next letter, if you wish to send soon. In the mean time, you can send your smaller forms by letter.

In the matter of the Doublet which I offer you, the proposition is made supposing that you will not easily find one in this country. If you could get one in Boston, of course it would be better to do so.

Respectfully yours,

M. A. CURTIS.

HILLSBOROUGH, N. C. Nov. 9th, 1857.

Dear Sir,

Herewith please find report on your last two envois. The specimens have been examined under the pressure of other duties, & a portion of them are not in very good condition;—but most of them are given with confidence. I was anxious to report them before entering upon a journey which I am about undertaking to Alabama, as I desire, upon my return in a few weeks, to come with free hands upon a large collection of Cuban Fungi just sent me by C. Wright. These will keep me busy for a while, so that I shall hardly be able to do much more for you before Jany. next. This, however, need not prevent your sending in the meanwhile, if you choose. I will examine your specimens as soon as I can.

Such Genera as *Sphaeropsis, Septoria,* &c. have so many forms, & are perhaps only secondary states of other Genera, that it is an empirical business to impose names upon them. *Pestalozzia* & *Phoma* form many uncertain species. Hence you see no specific names of these in the list.

In haste—yours respectfully

M. A. CURTIS.

[No date]

Dear Sir,

I have managed to find time to put up a few Fungi by way of support to the Doublet, & to make a rough kind of sketch of my Doublet stand,—all which are herewith enclosed. You had better ascertain what the maker will charge for constructing such a stand, before making a bargain with him. Those not accustomed to this sort of manufacture might charge $10.–$15. A Boston man, & the only one I could hear of in the City, six years ago,

who could work in brass, would not undertake such a thing for me under the latter sum. You can buy such a stand (the upper arm only altered to receive the Doublet) with all the appurtances of a Compound Microscope for $8.–$10.—Mine is of that kind. The thing I have sketched should not cost over $4 or $5.

The Doublet has a focal distance of not more than one line. The glass slides I use I have generally made myself out of bits of clear window-glass, cutting them about 1½ in. long & ½ in. wide.

A small bit of the hymenium of the Fungus is taken with the point of a sharp knife & macerated in a drop of water upon the slide—mashing the piece so as to be sufficiently disintegrated & mingled with the water that the mass may pass under the lens without any portion of it touching the lens. The field of vision with this Instrument is so limited, that it is only suited to the examination of objects invisible to the naked eye. It requires a firm stand & a nice adjustment.—I have given you a view of the fruit of Peziza ollaris, as it appears under this Doublet, magnified about 250 diameters. You could not see this fruit at all with the naked eye. I have never used any instrument but this.—You must always keep it protected from dust when not in use. This would gradually scratch & deface the Lens. If anything gets upon the lens, wipe it with a soft silk handkerchief free from dust, having previously blown off whatever the breath can remove.

Very truly yours,

M. A. CURTIS.

THE LAST LETTER

A number of the Curtis letters in the Blake correspondence are omitted because they add little to the knowledge already in hand regarding Curtis. It appears fitting, however, to include the closing paragraphs of the letter with which the correspondence itself ended and which shows that however little Curtis talked or wrote regarding politics[4] he was in that as in other respects a close observer.

HILLSBOROUGH, N. C. Feb. 21 '61.

Dear Sir:

. . . . . .

It is interesting to read your notes of a Maine Winter. I was raised in the Western part of Massachusetts and know very well what snow and ice and a low temperature are; but having now enjoyed a sub-tropical climate for 30 yrs. I have no desire to try a boreal Winter again. We have as cold weather here as I can enjoy, for we can freeze to death in Carolina, though not quite so quickly, as in Maine. Four years ago the thermometer here stood at 16° below Zero and snow (several feet deep) lay for weeks on the ground. But that was extraordinary. This winter we have had but one snow, and no ice has formed for packing away. This again is unusual,

[4] See also in this connection—Stevens, N. E., Two Southern Botanists and the Civil War. Scientific Monthly **9**: 157–166. 1919.

for we generally put up ice here for summer use. Violets, crocuses, & hyacinths are now blooming in my yard, & the elms are beginning to put out their blossoms.

You allude very delicately to the political state of affairs but I do not care to respond or correspond on that distressing subject. The all-wise ruler chastises as well as blesses, & I am sadly afraid that we are to be terribly afflicted for our national sins of worldliness, boastfulness, self-reliance & various acts of injustice. The 2nd Psalm and the Sermon on the Mount are what I generally recommend now for reading, more than ever before, & more thoughtfully. A very few days will, I suppose, bring matters to a crisis. If the incoming administration attempts coercion, whether it be legal & right or not it will unite the whole South as one man in resistance. The only possible chance of a restoration of the Union is just to let the seceding States alone. A little spark now thrown into such combustible material as the South presents, will kindle a blaze the like of of which was never before seen. Is not almost any sacrifice desirable that will avert the catastrophe. The Border States, so called, of the South, i.e. all the Slave States north of the seceded ones, have a large majority in favor of preserving the Union, & yet one impolitic act of Pres't Lincoln will throw them all out. God help us."

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

FUNGICIDAL VALUE OF SOME COMMON DYES AGAINST DERMATOPHYTIC FUNGI

ADELIA MCCREA

During the past twenty years, a considerable amount of work has been done relative to the value of dyes in bacteriological and, more recently, in mycological studies on the prevention or control of growth of the organisms concerned. In order to correlate the results, it seems desirable to give a brief resumé of the purposes and findings of some of these various workers.

In 1912 Churchman (**3**) published an extensive study on the selective bactericidal action of gentian violet, comprising over 300 strains of 137 species. He found the gentian violet positive and negative reactions far more definite and constant than reactions to the Gram stain but concluded that the effect on growth should be considered bacteriostatic (a term which he seems to have introduced) rather than bactericidal. Two years later, 1914, Krumwiede and Pratt (**7, 8**) studied 30 strains, covering all types of pathogenic bacteria, in relation to 40 samples of dyes, stressing especially the green dyes. They confirmed Churchman's findings as to the inhibition of most Gram positive bacteria but the Gram negative organisms grew freely as a rule. However, several of the green dyes showed marked selective action among the typhoid-colon group, suggesting their usefulness in enrichment of the typhoid-paratyphoid species. Kligler (**6**) in 1918 reported the inhibiting effect of 28 compounds on 2 Gram positive and 7 Gram negative organisms. As was true for the previous workers, he found the most marked selective action in the dyes of the triphenylmethane group, such as gentian violet, malachite green, etc., which are much more toxic for the Gram positive organisms than for Gram negative types. Ten years later, 1928, Mallman, Thorp and Semmes (**12**) tested gentian violet, basic fuchsin, crystal violet, acriflavine and brilliant green for selective action in paratyphoid

types but found only brilliant green to be satisfactory. They stressed the importance of the *source* of the dyes used, as great variation is found in products from different manufacturers. On this point the writer is able to confirm their statement and extend it to the work on fungi reported herein.

Several contributions have been made on the *fungicidal* potency of aniline dyes. After gentian violet had been shown to be so active against Gram positive bacteria, Farley (**5**) in 1920 tested its usefulness as a restraining agent in his work on isolation of mold pathogens. He reasoned that if the many Gram positive bacteria could be inhibited by a dye that would permit normal mold growth, isolation of pure species would be facilitated. He found that, although molds in general are Gram positive, they are much more resistant to gentian violet than are Gram positive bacteria. Thus he was able to grow normal cultures of 50 species of *Actinomyces, Sporotrichum* and " ringworm " fungi on media containing sufficient gentian violet to inhibit troublesome bacterial growth. Castellani (**1, 2**) 1928, 1929, reported the use of a solution of carbol-fuchsin in combatting certain types of skin infection by fungi. In most of his cases the responsible organism was not isolated, or at least is not given, but he has had considerable clinical success with this dye. The writer has been unable to confirm *in vitro* or on guinea pig lesions any appreciable value for this formula against *Epidermophyton rubrum.* Coons (**4**) 1927 and Leonian (**9**) 1929, found that malachite green decidedly inhibits development of *Fusarium* species, the latter having tested this dye upon 220 different cultures of *Fusarium,* only three of which made any growth at 1–10,000 while many were inhibited at 1–500,000. Leonian (**10**) also in 1930 used malachite green as an aid in studying *Phytophthora* species. He records a grouping of the strains by means of their differential growth upon media containing malachite green in dilutions up to 1–16,000,000 and holds that, despite the difficulty due to borderline reactions the dye is nevertheless useful in defining groups of species or strains within the genus *Phytophthora.* In 1932 Leonian (**11**) again tested the fungistatic potency of malachite green, and crystal violet as well, on 26 cultures of skin fungi of the genus *Trichophyton,* his object being mainly to study the effect of position of inoculum. Despite

uniform conditions, results were quite erratic and the conclusion was drawn that such dies are of doubtful value in identification work with *Trichophyton* types.

For several years the writer has been working with species of the four chief dermatophytic genera—*Trichophyton, Epidermophyton, Microsporum* and *Achorion*—in an attempt to find an ideal fungicide. Many substances have been tested for both fungistatic and fungicidal properties but the work herein given concerns only three species of fungi and a group of five of the most significant aniline types tested.

So far as the *fungistatic* power of these dyes is concerned, it may be disposed of briefly because in no case was the fungistatic limit widely separated from the fungicidal. In marked contrast to results with bacteria, it appears that unless a substance is capable of killing the fungus (fungicidal) it does not exercise any marked antiseptic effect. This was determined by sub-culturing, at suitable intervals after planting by fungistatic method, to determine whether the inhibition was merely stasis or whether killing action had occurred.

The fungicidal test method used in this work was published by the writer (**13**) in 1931, hence will not be given here in detail. Suffice it to say that painstaking effort is exercised to secure uniformity of physical conditions, medium, reagents, spore suspensions and all other factors capable of standardization.

The pathogenic organisms used were *Trichophyton interdigitale* Priestly and *Epidermophyton rubrum* Castellani. As a check, *Aspergillus niger* van Tiegham was used because it is usually quite resistant to inhibiting substances—which may account in part for its status as a "laboratory weed." The table shows the relative susceptibility of these organisms to the dyes tested. Several points are apparent from the results which may be summarized as follows:

1. The "green dyes," malachite green and brilliant green are greatly superior in potency compared with any others studied.
2. These two dyes are of equal value as fungicides.
3. They show decidedly a selective action on the organisms tested, *A. niger* and *E. rubrum* being considerably more resistant than is *T. interdigitale*.

4. Selective action is also shown by aniline violet which in concentrated solution kills *T. interdigitale* in 5 minutes but fails to kill *E. rubrum* or *A. niger* in intervals up to and including an hour.

5. Neither gentian violet nor basic fuchsin show any significant fungicidal power against these three organisms, in any practicable dilution.

COMPARATIVE FUNGICIDAL VALUE OF FIVE COMMON ANILINE DYES

	Aniline violet	Fuchsin basic	Gentian violet	Brilliant green	Malachite green
Trichophyton interdigitale	1–500 Kills in 5 minutes.	Does not kill.	Does not kill.	1–75,000 Kills in 1 minute.	1–75,000 Kills in 1 minute.
Epidermophyton rubrum	Does not kill.	Does not kill.	Does not kill.	1–10,000 Kills in 1 minute.	1–10,000 Kills in 1 minute.
Aspergillus niger	Does not kill.	Does not kill.	Does not kill.	1–10,000 Kills in 1 minute.	1–10,000 Kills in 1 minute.

RESEARCH LABORATORIES, PARKE, DAVIS AND CO.,
DETROIT, MICHIGAN.

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LONGEVITY OF MERULIUS LACRYMANS IN WOOD DESTROYED BY ITS GROWTH

ADELIA MCCREA

This note pertains to the persistence of life of *Merulius lacrymans* in wood destroyed by the growth of the fungus. Perhaps it should be stated at once that, although this species is herein termed *M. lacrymans* in the sense of American usage, it is doubtless *M. americanus* according to Burt's (1) classification.

In April, 1928, two specimens were received for study: (*a*) a piece of wood from the body of an automobile and (*b*) a fungus growing on the cloth upholstery of the same car. The car was one of the finest makes and had been in use between four and five years. It was involved in only a minor collision, but the body crumbled, due to destruction of the wood under the cloth.

At that time, the "mold" stage of the organism could be obtained readily from both the wood and the sporophore and, under proper conditions, would produce fruiting bodies in a few weeks as would also bits of the wood if first moistened. Each specimen has been kept wrapped in parchment paper on a shelf in the laboratory and tested at irregular intervals, at least once a year. In 1930, both were still viable but since then no culture has been obtained from the fungus on the cloth. Recent attempts included preliminary soaking of the fragments but the sporophore appears to be definitely dead.

The wood, however, never fails to give good results of the mycelial (mold) form and appears to be singularly free from any other organisms. If a piece be crumbled over a moist agar plate, every bit of the dust seems able to grow and in due time, transferred to wood, to form new sporophores.

If we may assume that the fungus was present in the wood when the car was built, the history would cover a period of ten to eleven years for the wood and over five years at least for the sporophore which, however, has been "dead" the last two or

three years of that time. It thus appears that life persists considerably longer in the wood than in the fruit body, when both are in the same unfavorable situation. This would agree with Findlay's (2) statement that difficulty in germinating the spores is due to the fact that workers have used spores no longer fresh. Falck (cited by Findlay) is authority for the statement that Merulius spores may be viable after five years laboratory storage but so far the writer is unable to confirm this finding.

These specimens will be preserved and the history of the wood followed up at intervals in an attempt to learn how long the organism will remain viable.

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A DEVELOPMENTAL STUDY OF A NEW SPECIES OF OPHIODOTHELLA[1]

E. Sophia Boyd

(with plate 51 and 2 text figures)

INTRODUCTION

This organism has been observed growing on the leaves of *Vaccinium arboreum* Marsh, in Georgia and other Southern States, and, being a leaf parasite, it has proven especially productive in a study of the development of a peculiar type of perithecium. The asexual stage begins in middle summer and continues throughout the fall, followed by the development of a perfect stage, with perithecia in the mesophyll of the leaf, beginning the latter part of September. At frequent intervals, the infected leaves have been gathered and sectioned, and the development of the organism studied. Both stages are apparently new and are so described in this paper.

This type of organism has been placed in the Phyllachoraceae of the Dothideales on the basis of having no true perithecial walls, but the ontogenetic studies here show a definite wall which places this in the Sphaeriales. On the basis of examinations of type specimens the writer here transfers the genus *Ophiodothella* from the Dothideales to the Sphaeriales.

METHODS

Leaves were gathered first in late September when still living and on the host. Later they were collected on the ground in damp

[1] This investigation was carried on under the direction of Dr. J. H. Miller to whom the writer expresses her appreciation for the generous assistance and helpful supervision in this work. Also, she is much indebted to Dr. W. W. Diehl, United States National Herbarium, Dr. F. L. Stevens, University of Illinois, and Dr. D. H. Linder, Farlow Herbarium, Harvard University, who have contributed both material and information relative to this study.

places, at seven to ten day periods, until April, when mature asci were found.

Material was fixed in Flemming's fluid, both strong and diluted fifty per cent with water; form-alcohol; and Bouin's fluid. The Flemming's fluid penetrated poorly, fixing only the epidermal and outer mesophyll cells. It increased the brittleness of the leaf, making sections very difficult to cut. However, the leaves were already very brittle, as in most cases they were dead when gathered. The form-alcohol solution penetrated well, but caused slight plasmolysis of the mycelial protoplasm. After Bouin's fluid there was good penetration with less brittleness than after the other two fixatives.

Sections were cut from paraffin, and were stained with Haidenhain's iron-alum haemotoxylin and counter stained in safranin. Very clear definition of nuclear structures was obtained from the use of the iron-alum haemotoxylin, while the safranin made a good contrast stain for the mycelium.

DEVELOPMENT OF THE IMPERFECT STAGE

The first visible sign of infection in the leaf is a small, yellow spot ranging from 1–5 mm. in diameter. A transverse section of this spot showed the vegetative mycelium running parallel to the epidermis. It becomes densely packed in the mesophyll and epidermal cells as well as in the intercellular spaces. The hyphal cells are large, thick-walled, nearly cylindrical, and uninucleate. The mycelium is very much branched, spreading into the epidermal cells, and there, due to oxidation following exposure to air, the walls turn black and give the appearance of a solid black layer as viewed from the outside of the leaf. They do not coalesce to form a stroma; however the layer might be called a pseudoclypeus.

The conidial potential forms between the epidermis and the palisade cells, and is made up of a tangled mass of hyphae, which do not anastomose, but interweave to form a dense plectenchyma of very minute cells (PLATE 51, FIG. 1–2). The conidiophores arise on the upper side of this plectenchyma, between it and the epidermis. They are multicellular stalks which grow perpendicular to the epidermis, forming a parallel layer that resembles an ascal hymenium (PLATE 51, FIG. 1–2). In some cases, however, the

plectenchyma becomes umbonate in the center. The cells of the conidiophore are uninucleate, and the basal cells are much larger than the upper ones (PLATE 51, FIG. 3). The apical cell is constricted into a narrow beak, resembling, to some extent, the one found by Jones (**10**: *pl. 4, fig. 1*) in *Melasmia acerina* Lév. This tip grows into a long, hyaline, filiform spore, which matures after the epidermis has been ruptured. The conidia range from 28–58 $\times$ 2 μ; are continuous, and the cells are uninucleate (PLATE 51, FIG. 4).

The fruiting body is an acervulus and not a pycnidium, because the pycnidium, as described by Dodge for *Schizoparme straminea* Shear (**2**: *pl. 4*), starts as a stromatic ball and the conidiophores arise in the center. In the conidial fruiting body of *Ophiodothella Vaccinii* the conidiophores are borne on the surface of the plectenchyma, and not down in it as in a true pycnidium. There is no definite ostiolar cavity as in the pycnidium that Dodge shows, but there is an aperture formed in the epidermis, due to the swelling and upward growth of the conidiophores. The conidia are cut off from the conidiophores successively as in the pycnidium, and are extruded in enormous masses through the ruptured epidermis.

The leaves of *Vaccinium arboreum* are tardily deciduous, hanging on until late December. Conidial infections continue as long as the leaves are alive.

DEVELOPMENT OF THE PERITHECIUM

Archicarp: The perithecial primordium arises in the center of the mesophyll and originates from one or several coiled archicarps, which, at this stage, are seen in a tangle of vegetative threads, as in *Polystigma rubrum* (Pers.) DC. (**5**: *fig. 160*) and in *Poronia punctata* (L.) Fries (**5**: *fig. 184a*). The archicarp (PLATE 51, FIG. 5) is a multinucleate hypha, which becomes septate and elaborately coiled at the base. It consists of a definite basal portion and a long, slender thread, probably a trichogyne, that grows straight up through the leaf and protrudes through a stoma. In many cases, several archicarps are grouped together and, thus, several trichogynes may grow through one stoma. No antheridium was found, but there is a possibility that the conidia, which mature simultaneously with the development of the coil, are ho-

mologous with spermatia, as found in many of the Hypocreales and Sphaeriales. According to Gwynne-Vaughan (**5**: 131), the more complex ascomycetes have antheridia that become detached and single cells function as spermatia; while on the other hand, probably more primitive forms, including all the Plectascales and many forms in the Pezizales, have non-deciduous antheridia. The conidia of *Ophiodothella Vaccinii* show marked resemblance to the spermatia of *Polystigma rubrum*, which are curved filiform cells, containing single elongated nuclei. A trichogyne is present in both, but no relation has been seen between it and the conidia of the one and the spermatia of the other. Higgins (**9**: 428) shows spermatia functioning in *Sphaerella bolleana*. Thus, it is possible that the spermatia in *Polystigma rubrum* are functional, but this has escaped the notice of observers, and judging from related forms in the Sphaeriales, the conidia of *Ophiodothella Vaccinii* probably at one time possessed spermatia similar to the vegetative cells that now function as conidia.

A basal cell in the archicarp continues to enlarge and is seen to possess two very large nuclei (PLATE 51, FIG. 6). This is an oögonial cell in the conception of Gwynne-Vaughan (**5**: *fig. 118–119*). It comes to lie in the center of the developing perithecial primordium. The binucleate condition in the oögonium arises either apogamously by the union of cells in the coil, or from a spermatial nucleus from the apex of a trichogyne.

In the cells of the thread-like trichogyne many very minute nuclei were found. These were so small that they may have been chromatin particles of disintegrating nuclei.

The two nuclei in the oögonium appear to pair and then divide conjugately passing into branches (PLATE 51, FIG. 7) from this cell, in the same manner as Claussen (**6**: 333) found in *Pyronema confluens* Tul., and the writer finds no evidence of a fusion at this point as Harper (**5**: *fig. 108*) observed in *Phyllactinia corylea* (Pers.) Karst., and Gwynne-Vaughan (**5**: 175) in *Pyronema confluens*.

Development of the perithecial wall: Below the oögonial region in the archicarp branch many very fine hyphae arise, completely surrounding the archicarp and proliferating tangentially until a globose perithecial primordium is formed. The peripheral cells are

small and uninucleate. By continued growth a definite wall is laid down (PLATE 51, FIG. 6–7). This is a perithecial wall as defined by Miller (**11**: 194) as being characteristic in the Sphaeriales: ". . . the specialized tissue which arises from the archicarp, and from the beginning encloses the ascigerous centrum." This same idea of a perithecium is later set forth by Graff (**7**: 244) when he says: "Properly the perithecium is a product of the gametophytic stalk cells from which the sexual reproductive organs also develop, and is an enclosing protective envelope whose initiation is evidently closely correlated with the formation of these organs."

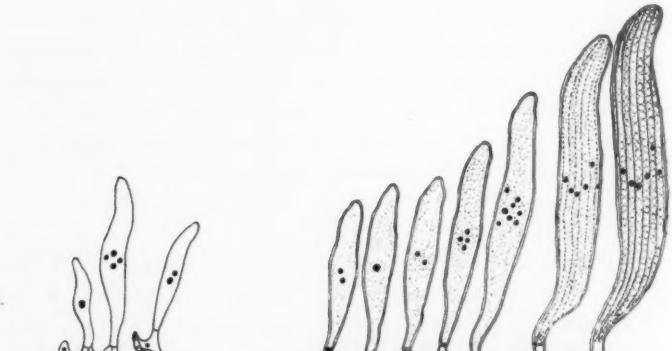

FIG. 1. a, Development of ascal hooks; b, Nuclear divisions in an ascus and formation of eight ascospores.

The wall cells, expanding peripherally, produce a rupture in the center. The inner wall cells now become very much flattened tangentially, and give rise to many sterile threads, which (PLATE 51, FIG. 8) grow toward the center of the perithecium. They are thin, thread-like, branched filaments with free ends, and so could definitely be called paraphyses.

The points of the perithecium facing the two epidermal layers now grow outward, the cells proliferating until the epidermis is ruptured on both sides. Thus two definite ostiola are formed, and the paraphyses developing in these beaks are very small and slender

and should be termed periphyses, such as Emmons and Dodge (**3**: *pl. 27, fig. 3*) show for *Microascus trigonosporus* Dodge. This now is a perithecium with a specialized wall, but is peculiar in the possession of two ostiola which permit the escape of ascospores from both sides of the leaf. The two ostiola are not always directly opposite so that several sections of the same perithecium have to be seen to observe both of them.

The only other forms that the writer has seen having two ostiola protruding through both sides of the leaf are *Catacauma biguttulatum* Theissen (**19**: *pl. 6, fig. 1*) and *Diachora onobrychidis* (DC.) J. Müll. (**4**: *fig. 248f*).

In the mature perithecium (PLATE 51, FIG. 8, and TEXT FIG. 2) the equatorial part of the wall becomes much thickened, while the wall of the ostiolar neck is very thin.

Origin of ascogenous hyphae: When the perithecium expands, rupturing the center, the archicarp with many branches coming from the oögonial region, comes to lie along the wall. In all longitudinal sections of perithecia examined, two such systems were found directly opposite each other in the perithecium, giving rise to two groups of asci. The cells coming off of the oögonial region form the ascogenous hyphae. These hyphae (PLATE 51, FIG. 8) are now found in a tangle along the wall and intermingle with numerous paraphyses. Their tips point inward as opposed to those of *Microascus trigonosporus* described by Emmons and Dodge (**3**: 331) as growing radially outward.

The archicarp of *Ophiodothella Vaccinii* gives rise to ascogenous hyphae before disintegrating, instead of disintegrating and then having the ascogenous hyphae arise from the wall cells as described by Blackman for *Polystigma rubrum* (**5**: 214).

Development of asci and formation of ascospores: The ascogenous hyphae branch freely and give rise to hooks (TEXT FIG. 1*a*), the penultimate cell developing into the ascus, in the same manner as shown by Claussen (**6**: 334) for *Pyronema confluens*. The asci form an equatorial belt on the inner wall and point toward the center (TEXT. FIG. 2), as Müller (**4**: *fig. 248f*) shows for *Diachora onobrychidis*. Theissen (**19**: 435) attempts to prove that Müller's conception (**12**: 346) of the origin of an equatorial belt of asci is incorrect, and he maintains that the asci form all over

the lower half of the locule, as in the normal situation, and that it is only later that the floor of the locule grows out into another ostiolum.

The writer has not examined specimens of *Diachora onobrychidis,* but in *Ophiodothella Vaccinii* the asci do arise in equatorial belts, and both ostiola develop simultaneously.

Two nuclei are cut off in the penultimate cell of the ascogenous

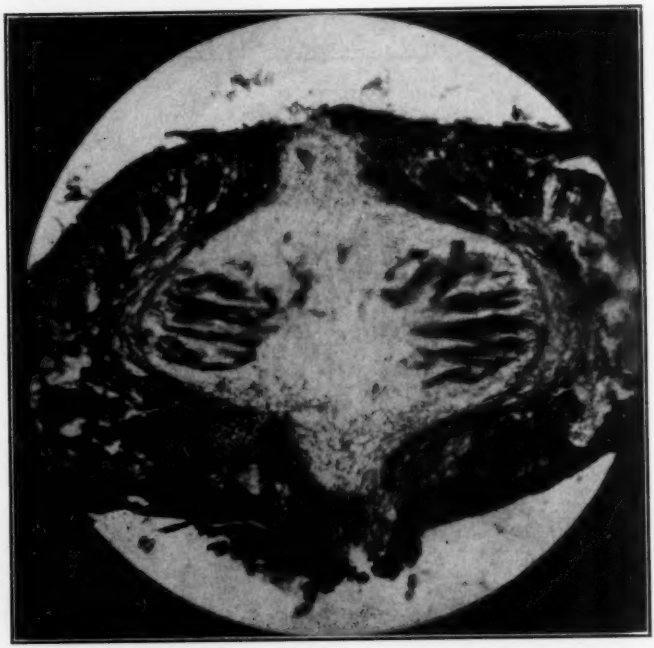

FIG. 2. Longitudinal section through a mature perithecium, showing equatorial belts of asci and two ostiola.

hook, the young ascus growing out toward the center of the perithecium. After fusion, reduction division takes place, followed by a mitotic division, and eight small nuclei come to lie in the center of the ascus (TEXT FIG. 1b). The ascospores are not cut out along lines radiating from a beaked nucleus as shown by Harper (**5**: *fig. 111*) in *Phyllactinia corylea* and many other Ascomycetes, and

by Schultz (**14**: 310) for *Peziza domiciliana* Cooke. Definite lines running the entire length of the ascus, are laid down. At first these appear to be vascular channels, but as the ascus matures, distinct membranes are formed, delimiting extremely long, filiform spores with one nucleus in the center of each. The ascospores are extruded through a small pore at the apex of the ascus.

TAXONOMIC RELATIONS

This organism has been placed in various herbaria of the country under the name of *Rhytisma Vaccinii* (Sw.) Fries: vide Ravenel Fungi Am. Es. *759,* Fungi Carol. *52,* Ellis, N. Am. Fungi *673* and de Thümen's Myc. Univ. *660.* This was due to a mistaken interpretation of *Rhytisma Vaccinii.* The Schweinitz type, according to Diehl in litt., is a true *Rhytisma* and is not a pyrenomycete. This mistake has probably been due to the fact that *Ophiodothella Vaccinii* forms the conidial stage with a macroscopic appearance very much like *Rhytisma punctatum* (Pers.) Fries. Also the perithecia here do not develop until spring, and on dead leaves lying on the ground, and therefore, probably have been overlooked.

The systematic position of this fungus lies within the Sphaeriales. The reasons for this are that here there are perithecia in the strict sense of the word. There is a specialized perithecial wall arising from the initial coil, definite ostiola with periphyses, and definite paraphyses with free ends. On the other hand the separation characters of the Dothideales are one or more locules in a stroma with no specialized wall enclosing the ascigerous centrum, no ostiolum, only a break in the upper portion of the stroma, no periphyses, the asci growing up in pseudoparenchyma or among threads attached to the roof of the cavity as well as the base.

The correct family within which this fungus should be placed cannot be determined at this time. The family separations in the Sphaeriales, according to Lindau (**4**: 386–387), are based on the position of perithecium relative to substrate or stroma, and amount and kind of stroma, all of which are environmental characters which can be brought forth within any one species. The writer will temporarily place *Ophiodothella* within the Clypeosphaeri-

aceae, because of the presence of a stromatic clypeus within the epidermis, and will await further revision of the order.

The genus *Ophiodothella* Höhn. is based on the species *Ophiodothis atromaculans* Henn., material of which has been loaned the writer by D. H. Linder. This genus is now in the Dothideales according to Theissen and Sydow (**19**: 611) and according to Shear and Clements (**16**: 293). Sections of this material show perithecia in the center of the mesophyll with true perithecial walls, definite ostiola penetrating the epidermis, with many fine periphyses, and very fine branched paraphyses within the ascal cavities. The ascospores are very long and filiform. The perithecia are not in a continuous stroma, but within the epidermis there are enough blackened semi-coalesced hyphae to be called a clypeus. Therefore the genus *Ophiodothella* must be removed from the Dothideales and placed in the Sphaeriales.

The genus *Scolecodothis* Theiss. & Syd. (**18**: 277) was made on the species *hypophylla* (Theiss) and according to the description is very closely related to *Ophiodothella,* if not identical. Shear and Clements (**16**: 92) placed both in the subfamily Phyllachorae of the Dothideales, and separate them only on: paraphyses present—*Scolecodothis,* paraphyses lacking—*Ophiodothella.* The writer finds that there are paraphyses in *Ophiodothella,* and in fact von Höhnel (**19**: 612) in the diagnosis says "paraphysen dunnfadig, sparlich," which would place these genera together according to Shear and Clements. Theissen and Sydow (**19**: 177) however, separate *Scolecodothis* and *Ophiodothella* on the fungus being between the epidermis and the palisade for the former, and lying in the mesophyll for the latter. If this character is constant then *Scolecodothis* should be in the same family with *Ophiodothella,* but not synonymous. The writer examined part of the type, but found only the conidial stage.

Another possible synonym for *Ophiodothella* is *Scolecodothopsis* Stevens (**17**: 183) based on the species *Ingae* Stevens, material of which has been loaned the writer by Stevens. The same generic characters as those of *Ophiodothella* were discovered here and the name has been reduced to synonomy. Shear and Clements (**16**: 293) so cite it.

Chardon (**15**: 60) describes the genus *Clypeotrabutia* with char-

acters similar to those of *Ophiodothella*. His type species is *portoricensis* (Stev.). In the generic description, he says spores one-celled and hyaline, which would include spores of any shape. In his description of the species, however, he says spores filiform, which would of necessity place his genus as a synonym under *Ophiodothella*. Then later, Chardon (**1**: 269) describes new species with eliptical spores. From mycological precedent, forms with filiform spores have generically been separated from those with other shapes, so such a species is not cogeneric with the type of *Clypeotrabutia*, and this genus is listed below as a synonym under *Ophiodothella*.

Shear and Clements (**16**: 259) cite *Clypeotrabutia* as a synonym under *Causalis* Theiss. Theissen (**20**: 184), in an illustration of the type species, *Myrtacearum* Theiss., shows perithecia in an enlarged stroma resting on the palisade and becoming erumpent through the epidermis, which is an entirely different situation from *Clypeotrabutia*, in which the perithecia are not stromatic and lie in the mesophyll.

The presence of two opposite ostiola in the perithecium of *Ophiodothella Vaccinii* does not seem to be sufficient grounds for the creation of a new genus. J. Müller, however, did erect the genus *Diachora* from a *Phyllachora* species which had opposite ostiola in the perithecia, but Theissen (**19**: 435) does not think that this is sufficient for a separation. Also, Shear and Clements (**16**: 293) refuse to recognize the genus *Diachora*.

DESCRIPTION OF THE ORGANISM

OPHIODOTHELLA Höhn., Frag. Myk. no. 630, 1910.

Scolecodothopsis Stevens, Ill. Biol. Monog. **8**: 183, 1923.

Clypeotrabutia Chardon, Sci. Surv. Porto Rico **8**: 60, 1926.

Ophiodothella Vaccinii sp. nov.

Type: no. *7091*—Univ. Georgia Crypt. Herb.—April, 1933, Athens, Georgia. Cotypes deposited in Nat. Herb., Washington, D. C.; Harvard Crypt. Herb.; Kew Herb.; The New York Botanical Garden.

Plate 51, text figures 1–2.

Maculae epiphyllae juvenes in foliis vivis 1–5 mm. diam. orbiculares non zonis cinctae margine aurantiaces; maturae in foliis deciduis vel mortuis

3–13 mm. diam. orbiculares plus minusve confluentes epidermide nigrescentae speciem stromatis facientae, mycelio fusco in utrisque stratis epidermidis pseudoclypeum formante; compluribus peritheciis in medio mesophyllo immersis 336 μ diam., 168 μ alt., duobus ostiolis, altero supra altero infra protrudente; ostiolo minute papillato puncticulato 70 μ diam., 98 μ alt., ex epidermide protrudente; parietibus perithecii 28 μ crassis ad aequatorem, 6–14 μ crassis ad aliam partem, investis ascis praeterquam in ostiolis; ascis ad centrum perithecii directis, cylindricis parietibus tenuibus, iodino +, subsessilibus 75.6–98 μ × 11.2 μ, octosporis; ascosporis filiformibus guttulatis hyalinis curvatis cum ex asco liberatis, apicibus obtusis 67.2–97.4 μ × 2.8 μ; paraphysibus multis filiformibus ramosis septatis, apicibus liberis.

Acervuli subepidermales in maculis laxe dispersi et elevate; strato conidio 358.4–476 μ diam., 84 μ crasso e strato parallelo conidiophororum oriundo; conidiis filiformibus plus minusve curvatis hyalinis continuis 28–58 μ × 2 μ ab epidermide rupta extrusis.

In foliis *Vaccinii arborei,* Res publica coniuncta meridionis orientalis; peritheciis in Martio et Aprili maturantbus, conidiis in aestive et autumno.

Spots epiphyllous; young spots on living leaves, 1–5 mm. in diameter, orbicular, not concentric, orange-colored margin; mature spots on fallen or dead leaves, 3–13 mm. in diameter, orbicular, more or less confluent, epidermis becoming black giving appearance of stroma, dark mycelium in both layers of the epidermis forming a pseudoclypeus; several perithecia central in mesophyll, 336 μ in diam., 168 μ high, with two ostiola, one protruding through the upper epidermis, the other through the lower; ostilum minutely papillate, minutely punctate, 70 μ in diam., 98 μ high; perithecial wall 28 μ thick at the equator, 6–14 μ thick at other part, covered with asci except in ostiolar cavities; asci directed toward center of the perithecium, cylindrical, thin-walled, iodine reaction plus, subsessile, 75.6–98 μ × 11.2 μ, eight-spored; ascospores filiform, guttulate, hyaline, curved when free from ascus, obtuse ends, 67.2–98.4 μ × 2.8 μ; paraphyses many, filiform, branched, septate, free ends.

Acervuli subepidermal, scattered and raised in spots; conidial layer 358.4–476 μ in diam., 84 μ thick, arising from parallel layer of conidiophores; conidia filiform, curved, hyaline, continuous, 28–58 μ × 2 μ, extruded through ruptured epidermis.

On *Vaccinium arboreum,* southeastern United States; perithecia maturing in March and April, conidia in summer and fall.

SUMMARY

1. The fungus on *Vaccinium arboreum* often determined in various herbaria as *Rhytisma Vaccinii* is a true pyrenomycete belonging to the genus *Ophiodothella.*

2. The developmental study of this form shows that it arises from a coil, the basal part of which is binucleate, the upper part forming a probable trichogyne of multinucleate cells. A definite wall forms, and there is a special feature in the two ostiola breaking out on opposite sides of the leaf. The ascal layer lies in an equatorial belt on the inner face of the perithecium which is different from the usual situation in the pyrenomycetes.

3. Members of this genus and related forms have been placed in the family Phyllachoraceae of the Dothideales, but the presence of the true perithecial wall, ostiolar formation, and paraphyses is indicative of their relationship in the *Sphaeriales,* and the genus *Ophiodothella* is here transferred to the family Clypeospaeriaceae of the Sphaeriales.

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EXPLANATION OF PLATES

PLATE 51

Ophiodothella Vaccinii. Fig. 1, Transverse section of the host leaf, showing a mature acervulus and feeding mycelium; 2, Portion of fig. 1 enlarged; 3, Conidiophore; 4, Conidium; 5, Longitudinal section of several coiled archicarps located in the middle of the host leaf, with trichogynes protruding through a stoma; 6, Longitudinal section of a young perithecium showing the oögonium and another cell of the coil in binucleate condition; 7, Longitudinal section of an older perithecium showing positions of branches from the oögonium and formation of the central cavity; 8, Longitudinal section of a perithecium showing the thickening of the equatorial parts of the wall and proliferation of the wall tissue at upper and lower extremities to form the two ostiola. Also, ascogenous hyphae are seen in an equatorial wall belt, intermingling with paraphyses.

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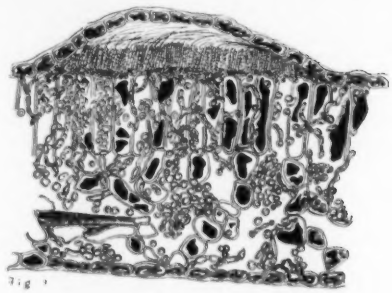

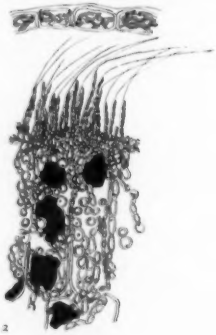

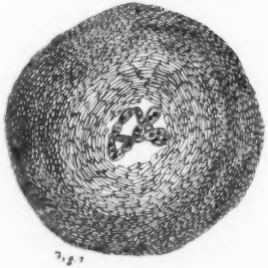

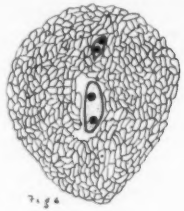

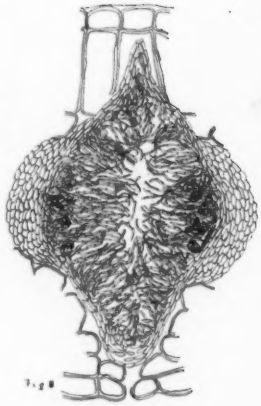

OPHIODOTHELLA VACCINII

NOTES AND BRIEF ARTICLES

The Myxomycetes

The volume under the above title by Thomas H. Macbride and G. W. Martin was issued on March 21, 1934, although the senior author quietly passed away a few days before the book was off the press. It is quite fitting, therefore, that an announcement of this new work and the portrait and brief biographical sketch of the senior author should appear in the same issue of Mycologia. The work is based on "North American Slime-Moulds" the second edition of which was published in 1922. To those familiar with this work scarcely more than an announcement is necessary.

The work has been completely revised and enlarged, consisting of 339 pages and 21 plates. Of necessity the enormous task of revising and completing this volume has fallen heavily upon the junior author and he should be highly commended for his efforts in putting through this task. The work is published by the Macmillan Company, as were the former volumes of North American Slime-Moulds.

Manual of the Rusts in United States and Canada [1]

Students of the plant rusts (Uredinales) have been anticipating for some years the appearance of Doctor Arthur's manual of this group upon which he has been working since the publication of "The Plant Rusts" (John Wiley & Sons, 1929). The reviewer believes that they will not be disappointed with the present volume.

Few groups of organisms present such difficult taxonomic problems as do the rusts. To a considerable extent this is due to their condition of obligative parasitism, regarding which our notions have materially changed since the passing of the last century. We have now come to recognize in part the effect of parasitism in producing morphological retrogression while at the same time physiological specialization is evolving many racial characteristics.

[1] The Science Press Printing Co., Lancaster, Pa., 1934. Price $6.00.

I consider the taxonomy of the rusts to have passed through five rather distinct periods. In the *first period* the basis of classification was strictly monomorphic, the importance of the life-cycle being unknown. Each spore-form was considered a distinct species.

The *second period* was initiated with the establishment of polymorphism by Tulasne and followed by the recognition of the interesting condition to which deBary gave the term heteroecism.

The *third period* came with the recognition of the relationship of the rusts with the Basidiomycetes and the acceptance of the teliospore as the significant stage in the life-cycle. As a result, Dietel's arrangement in Engler and Prantl's Pflanzenfamilien has been generally accepted.

In the meantime Schroeter (1889) introduced the biologic viewpoint, emphasizing the various cycles represented in the genus. This scheme enlarged upon by Arthur in 1906 as the basis of the taxonomic treatment presented in the North American Flora constitutes the *fourth period*. In this work Arthur stressed both biologic and morphologic characters, features which proved most stimulating to researches in the rust fungi.

Along with these developments has come the extensive studies in specialization by Eriksson, Klebahn, Stakman and others. These researches, together with Craigie's discovery of the function of the pycnium and the studies of Jackson and others on the origin of life cycles have served to emphasize the physiologic side of the problem and complicate very greatly the presentation of any natural system of classification.

Gradually the concept has arisen that the species consists of two stages of development (haploid and diploid). With the recognition of this fact and the knowledge that the morphology of the dominant spore form, the teliospore, is a fairly stable character, Dr. Arthur has brought together in this manual a classification which at least approaches what may be considered a natural arrangement.

With the appearance of this manual the *fifth period* is initiated. In this work Dr. Arthur has made a very definite attempt to bring together all species or forms of rusts which show degrees of relationship other than that indicated by hosts. This has necessi-

tated reducing some of the older recognized genera to sectional rank, as well as some recognized specific names to varietal rank. All this involves the nice question of species limitations. How distinct must a variation in life cycle, or in the more detailed morphological features become in order to be recognized as a generic or specific entity? The final answer has not been given but graduate students will surely find in this treatment of the rusts many problems which are stimulation to further researches.

The manual contains several unique aids for taxonomic work. A list of the names of authors of both rust species and host species is given together with their date of birth, and death dates in the case of those deceased. There is also an explanation of terms and usage, and a glossary which will be found helpful to the beginner.

Two families are recognized: Melampsoraceae with four tribes and fifteen genera, and Pucciniaceae with three tribes and seventeen genera. The descriptions are curtailed in comparison with those in the N. A. F. but sufficiently full to enable identification. Of great value are the illustrations prepared by Mr. George B. Cummins who has exhibited much skill and discrimination in presenting the outline drawings of the critical spore stages in nearly every species.

It is ardently hoped that this work will be only the beginning of a series of similar illustrated manuals of the different important groups of fungi.

C. R. ORTON

INTERPRETATION OF RULE 49BIS [1]

The fundamental purpose of the International Rules of Botanical Nomenclature is to secure a reasonable nomenclatural stability and uniformity of usage. The Rules were published and became effective in 1912 following the International Botanical Congress of 1905 and 1910. Rule 49*bis* applies solely to the Ascomycetes and Uredinales. What follows in this article pertains to the Uredinales, without in any way involving the Ascomycetes. The Rule

[1] Contribution from the Botany Department, Purdue University Agricultural Experiment Station.

has not resulted in general acceptance, partly because of what are accounted inherent defects and partly from diversity of interpretation.

It is well in presenting a subject to clearly understand the significance of the terms employed. The Rule makes use of the terms "state," "stage" and "form," whose exact meaning is not necessarily apparent. The Rule, in so far as it relates to the Uredinales, reads thus:

"Art. 49*bis*. Among fungi with a pleomorphic life-cycle the different successive states of the same species (anamorphoses, status) can bear only one generic and specific name (binomial), that is, the earliest which has been given to the state containing the form which it has been agreed to call the perfect form, provided that the name is otherwise in conformity with the rules. The perfect state is that which ends in the teleutospore or its equivalent in the Uredinales. Generic and specific names given to other states have only a temporary value. They cannot replace a generic name already existing and applying to one or more species, any one of which contains the 'perfect form.'"[2]

To elucidate the Rule in dealing with the Uredinales it is well to remember that there are two *states* in every species of this order whatever its generic connection, the gametophytic or haploid, which bears aecidiospores, and the sporophytic or diploid, which bears uredospores and teleutospores. In the full life-cycle of a species there may be at times different *stages,* according to the kind of spores produced, as the aecidiosporic, uredosporic or teleutosporic stages. The body of spores evolved in any stage constitutes a *form*. In reduced species, especially the so-called short-cycle species, the two states are much curtailed, and some of the spore-forms may be suppressed, sometimes the aecidiospores, sometimes the uredospores, or in some species both.

The rule clearly sets forth that "the perfect state is that which ends in the teleutospore," or to agree with previous wording, "contains" the teleutospore. By inference the "perfect form" must be that of the sori bearing teleutospores. Neither the wording of the rule nor the history of spore development excludes the uredo-

[2] Briquet, John. Regles Internationales de la Nomenclature Botanique, 110 pp. June, 1912.

spore, a product of the sporophyte, and a normal part of the "perfect state," although the aecidiospore, a product of the gametophyte, is clearly excluded.

In applying the rule it should be borne in mind that the spores are not to be defined as to their structure, but only as to their origin or their mode of germination. Every permanent generic or specific name must be founded upon the "perfect state," which is equivalent to saying that it must be founded upon the sporophytic state, a state which bears uredospores and teleutospores. The test of a "form," in order to decide whether the name applied to it is to be considered a "permanent name" or only a "temporary name," must be either its origin or the germination of its spores or both, whatever the general appearance of the spores constituting the form may be.

If the definition of terms given at the beginning of this article be accepted, which the wording of the Rule logically requires, making the uredospore a product of the "perfect state . . . which ends in the teleutospore," the names applied to the uredosporic stage are not "temporary," but permanent, that is, acceptable, the same as those applied to the teleutosporic stage.

A common interpretation, however, makes the Rule exclude names from recognition in priority when applied to the uredosporic stage. This interpretation is not based upon the practice of uredinologists present or past, as a statistical study clearly shows, but can be traced to the impression held by many botanists, both mycologists and others, that as uredospores usually (but not always) function for the dispersal of the species they are of the nature of conidia,[3] and therefore should not be considered as belonging to the "perfect state."

Uredospores, however, are borne on sporophytic mycelium and are binucleate, a condition which is not generally true of conidia as ordinarily considered. The same sorus producing uredospores often produces teleutospores, either at the same time or later. If a species does not produce uredospores in the cycle of development, the teleutosporic sori may wholly or in part (*e.g.*, in *Puccinia veronicarum*) become pulverulent, and scatter their spores in the same manner and quite as effectively as any uredospores. True

[3] Gwynne-Vaughan, Fungi, 1922, p. 4.

conidia, as in the Ascomycetes and higher Basidiomycetes, are borne on the gametophytic mycelium and are haploid. Such spores do not occur in the Uredinales. The adaptation for rapid dissemination is not inherent in any one kind of spore. The fact that the uredospores play the rôle of rapid propagation does not entitle them to be called conidia.[4]

Not only is the assumption incorrect that uredospores are of the nature of conidia, but usage as shown by the publications of systematic uredinologists is opposed to such an interpretation of the Rule. The only recent work embracing all known species of the Uredinales from every part of the world is that of Sydows' Monographia Uredinearum. The work is accurately compiled, and the synonymy is especially complete. The names of species recognized are with rare exceptions those which are in general use.

It is interesting to note that out of 2333 species embraced in this work having teleutospores only 26 species are affected by the varying opinion regarding the uredospore, and that in every instance to exclude from priority the names applied to the uredosporic stage of these 26 species will require a change from the well established and generally accepted names of long standing and the substitution in most instances of unfamiliar names. Moreover, to invalidate uredosporic names would permit the changing of the specific names of more than 400 species now known only under the genus *Uredo,* when their teleutospores are discovered, a number to which many names are constantly being added.

It may be asked why make a change for so small a number as 26 names out of 2333 species; and as to the possible substitution of new specific names under the genus *Uredo* upon the discovery of teleutospores it may be asserted that no serious mycologist would intentionally do it. In answer, let it be distinctly understood that no change in the Rule is advocated. The Rule is to stand as it is. What is needed is a better understanding of the individual development of the rusts, and a clearer interpretation of the Rule. By so doing and accepting the Rule as it stands the 26 species referred to will have the specific names now in general use validated, no changes being required, and the specific names of 400 or more

[4] Gäumann, Vergleichende Morphologie der Pilze, 1926, p. 435; English translation by Dodge, 1928, p. 568.

species now only known in the genus *Uredo* made permanently acceptable. It is only by insisting that uredosporic names should not be recognized in priority that changes for the 26 names are required, and which permits changes for names now under the genus *Uredo*.

Following Rule 49*bis* is a paragraph giving "Examples." These pertain to the genera *Aecidium* Pers., *Caeoma* Link, and *Uredo* Pers., as they are used to designate different stages in rust development. It is not made clear that these three names have at no time been abandoned as generic names. They were first applied to stages by de Bary in 1863, who authorized at the same time the use of the terms aecidiospore and uredospore and also the wholly new term teleutospore. An error is made in stating that *Melampsora* (1848) is antedated by the use of *Uredo* to designate a state (1863). The names *Aecidium, Caeoma, Uredo* and some others, have by general consent long passed into synonmy as standard generic names, although for convenience they are retained as "form-genera." The paragraph following the Rule should be reworded to remove the confusion resulting from the use of names both for stages and for genera. *Caeoma* Link is a synonym of *Phragmidium,* and the names *Aecidium* and *Uredo* need to be placed in the list of *genera conservanda* as synonyms of *Gymnosporangium* and *Melampsora* respectively.

It may be well to call attention in this connection to the fact that a specific name is not invalidated by being first published under a wrong genus, even one not belonging to the Uredinales. Many early names were established under such non-rust genera as *Ascobolus, Lycoperdon, Tremella, Oidium, Sphaeria,* etc., to which might be added the sometimes misplaced genera *Aecidium, Uredo* and others.

Theoretically the name first applied to a rust in any stage of its development, whether of gametophytic or sporophytic origin, should be considered the accepted name of the species, as the writer exemplified in the 7th volume of the North American Flora, but in practice and to maintain stability in the nomenclature it has been found desirable to exclude aecidiosporic names, or any others applied to the gametophytic (sexual) state, from recognition in de-

ciding priority. This is now acceptable to nearly or quite all uredinologists and is in accordance with the intent of the Rule.

Although having no direct bearing upon the nomenclatural problem, it is interesting to note that it is the uredo-stage that supplies the common name "rust," and its equivalent in ancient times, by which this group of fungi is best known to the non-technical public. The uredo-stage has always been recognized as a characteristic feature of the Uredinales. In fact, no other fungi possess spores of like nature, although sometimes possessing spores having a superficial resemblance and erroneously designated as species of *Uredo*. It was the uredosporic stage of the rusts that suggested to Persoon in 1818 the name Urédinées for the group, which later became Uredineae, and finally Uredinales.

Conclusion

Rule 49*bis* of the International Rules of Nomenclature is written in involved phraseology, but when fully understood supports the general practice of systematic uredinologists, both past and present. The Rule excludes aecidiosporic names (of the gametophytic state), but includes uredosporic and teleutosporic names (of the sporophytic, or "perfect" state). This interpretation accords with the individual development of the rusts, and also promotes stability and uniformity of usage, which are the basic purposes in promulgating the Rules.

The examples cited, following Rule 49*bis*, are confusing, and not wholly accurate. They need to be rewritten.

Incidentally it may be pointed out that the uredosporic stage is the most characteristic feature of the Uredinales, and the one to receive popular recognition. Moreover, it is the stage which gives the only name by which the order has at any time been known.

J. C. ARTHUR

TWENTY-FOUR YEAR INDEX

The proposed Twenty-four Year Index of MYCOLOGIA is now in the process of printing and it is expected that this will be issued early in 1935. It is estimated that it will comprise approximately 275 pages. The index will consist of two parts:

1. An alphabetical list of authors with full titles of their papers arranged chronologically.

2. An index to genera and species and their subdivisions of the fungi including myxomycetes, bacteria, and lichens.

This index will be issued in two forms, in paper covers or in board covers with fabrikoid. The pre-publication price is $2.50 for the former and $3.00 for the latter. Any orders entered before December 31, 1934 will be filled at this rate. After January 1, 1935 the price will be increased $.50 on each. In placing orders be sure and indicate whether you wish your copy in paper covers or in board covers bound in fabrikoid.

OBITUARY

Dr. Karl Frederic Kellerman, for many years Associate Chief of the Bureau of Plant Industry, died August 30, at Washington, D. C. Dr. Kellerman, in addition to his administrative duties, directed the campaign for the eradication of Citrus canker and the phoney disease of peach. He was instrumental in establishing the Journal of Agricultural Research and served 11 years as chairman of its editorial board. He was appointed to the National Research Council in 1917 by President Wilson and later became a member of the Division of Biology and Agriculture. He was for a number of years a member of the Federal Horticultural Board and took an active part in furthering the work in plant quarantine carried on by this organization. Dr. Kellerman became Chief of the Division of Plant Disease Eradication in the Bureau of Entomology and Plant Quarantine in 1933. It will be remembered that he was the son of the late Dr. W. A. Kellerman, one of the founders of the Journal of Mycology and a mycologist of note.

JOHN A. STEVENSON